```
=> d his

     (FILE 'HOME' ENTERED AT 13:24:46 ON 30 JUL 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 13:25:29 ON 30 JUL 2004
L1      1222343 S KINASE?
L2      2111610 S CALCIUM OR CALMODULIN
L3        21392 S L1(4W)L2
L4           34 S HUMAN (A)L3
L5           28 DUP REM L4 (6 DUPLICATES REMOVED)
L6      6635055 S CLON? OR EXPRESS? OR RECOMBINANT
L7         5864 S L3 AND L6
L8         1850 S HUMAN AND L7
L9         1641 S RETINOBLASTOMAS
L10           1 S L8 AND L9
L11         331 S "375" AND GLYCINE
L12          64 S L6 AND L11
L13          35 DUP REM L12 (29 DUPLICATES REMOVED)
                E WEI M/AU
L14         806 S E3
                E DIFRANCESCO V/AU
L15         113 S E3-E4
                E BEASLEY E M/AU
L16         300 S E3
L17        1130 S L14 OR L15 OR L16
L18           1 S L3 AND L17
```

```
Connecting via Winsock to STN


Welcome to STN International!  Enter x:x

LOGINID:SSSPTA1652MXM

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  May 12   EXTEND option available in structure searching
 NEWS  4  May 12   Polymer links for the POLYLINK command completed in REGISTRY
 NEWS  5  May 27   New UPM (Update Code Maximum) field for more efficient patent
                   SDIs in CAplus
 NEWS  6  May 27   CAplus super roles and document types searchable in REGISTRY
 NEWS  7  Jun 28   Additional enzyme-catalyzed reactions added to CASREACT
 NEWS  8  Jun 28   ANTE, AQUALINE, BIOENG, CIVILENG, ENVIROENG, MECHENG,
                   and WATER from CSA now available on STN(R)
 NEWS  9  Jul 12   BEILSTEIN enhanced with new display and select options,
                   resulting in a closer connection to BABS
 NEWS 10  Jul 30   BEILSTEIN on STN workshop to be held August 24 in conjunction
                   with the 228th ACS National Meeting

 NEWS EXPRESS   MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
                MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
                AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
 NEWS HOURS     STN Operating Hours Plus Help Desk Availability
 NEWS INTER     General Internet Information
 NEWS LOGIN     Welcome Banner and News Items
 NEWS PHONE     Direct Dial and Telecommunication Network Access to STN
 NEWS WWW       CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:24:46 ON 30 JUL 2004

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 13:25:29 ON 30 JUL 2004

FILE 'EMBASE' ENTERED AT 13:25:29 ON 30 JUL 2004
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

FILE 'BIOSIS' ENTERED AT 13:25:29 ON 30 JUL 2004
```

COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'BIOTECHDS' ENTERED AT 13:25:29 ON 30 JUL 2004
COPYRIGHT (C) 2004 THOMSON DERWENT AND INSTITUTE FOR SCIENTIFIC INFORMATION

FILE 'SCISEARCH' ENTERED AT 13:25:29 ON 30 JUL 2004
COPYRIGHT 2004 THOMSON ISI

FILE 'HCAPLUS' ENTERED AT 13:25:29 ON 30 JUL 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'NTIS' ENTERED AT 13:25:29 ON 30 JUL 2004
Compiled and distributed by the NTIS, U.S. Department of Commerce.
It contains copyrighted material.
All rights reserved. (2004)

FILE 'LIFESCI' ENTERED AT 13:25:29 ON 30 JUL 2004
COPYRIGHT (C) 2004 Cambridge Scientific Abstracts (CSA)

=> s kinase?
L1 1222343 KINASE?

=> s calcium or calmodulin
L2 2111610 CALCIUM OR CALMODULIN

=> s l1(4w)l2
L3 21392 L1(4W) L2

=> s human (a)l3
L4 34 HUMAN (A) L3

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 28 DUP REM L4 (6 DUPLICATES REMOVED)

=> d 1-28 ibib ab

L5 ANSWER 1 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:869664 HCAPLUS
DOCUMENT NUMBER: 140:71493
TITLE: Human calcium-sensing receptor can be suppressed by antisense sequences
AUTHOR(S): Maldonado-Perez, David; Breitwieser, Gerda E.; Gama, Lucio; Elliott, Austin C.; Ward, Donald T.; Riccardi, Daniela
CORPORATE SOURCE: School of Biological Sciences, University of Manchester, Manchester, UK
SOURCE: Biochemical and Biophysical Research Communications (2003), 311(3), 610-617
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have evaluated the ability of an antisense cDNA sequence, directed to the amino-terminus of the human calcium-sensing receptor (CaR), to reduce the expression and function of an EGFP-tagged CaR (CaR-EGFP) in HEK293 cells. Confocal microscopy and Western blot anal. showed a significant and selective reduction of the expression of CaR-EGFP by the antisense construct. Measurements of changes in intracellular calcium induced by CaR agonists showed that CaR-EGFP function was significantly reduced by the antisense sequence, as was agonist-evoked phosphorylation of

extracellular signal-regulated protein kinases (ERK1,2). A sense construct directed to the same region of the receptor had no effect, confirming the specificity of the antisense construct. Our results indicate that a CaR antisense cDNA reduces both the expression and function of the receptor. In the absence of strong, specific pharmacol. inhibitors of CaR, the antisense approach will be helpful to elucidate contributions of the CaR to cell physiol.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:141072 HCAPLUS

DOCUMENT NUMBER: 139:49066

TITLE: Activation of S6K1 (p70 ribosomal protein S6 kinase 1) requires an initial calcium-dependent priming event involving formation of a high-molecular-mass signalling complex

AUTHOR(S): Hannan, Katherine M.; Thomas, George; Pearson, Richard B.

CORPORATE SOURCE: Trescowthick Research Laboratories, Peter MacCallum Cancer Institute, Melbourne, Victoria, 8006, Australia

SOURCE: Biochemical Journal (2003), 370(2), 469-477
CODEN: BIJOAK; ISSN: 0264-6021

PUBLISHER: Portland Press Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The mitogen-stimulated protein kinase p70 ribosomal protein S6 kinase 1 (S6K1) is a key enzyme in the regulation of cell growth and proliferation. Activation of S6K1 requires a complex, ordered series of conformational changes and phosphorylation reactions. While the role of sequential, multi-site phosphorylation has been extensively detailed, characterization of the priming step required to initiate this cascade has remained elusive. In the present study we show for the first time that this priming process is dependent on calcium. Calcium-dependent regulation of S6K1 did not specifically target Thr-229 and Thr-389, the key regulatory phosphorylation sites; rather, calcium chelation resulted in a global inhibition of S6K1 phosphorylation. Mutation of individual phosphorylation sites in the auto-inhibitory and hydrophobic domains to acidic residues (to mimic phosphorylation) yields a kinase that remains sensitive to calcium chelation, while the combined mutations alleviate the requirement for calcium. Furthermore, deletion of the C-terminal residues (398-502) also renders the kinase insensitive to calcium. We hypothesize that the initial calcium-dependent process is required to release an inhibitory interaction between the C- and N-termini of S6K1, thus allowing phosphorylation of these key domains. The requirement for this priming step can only be overcome by mutations mimicking the phosphorylation of both the auto-inhibitory and hydrophobic domains. We further propose that the priming event involves formation of a calcium-dependent protein complex that releases the interaction between the N- and C-termini. S6K1 is then accessible for activation by the kinases that target the known regulatory phosphorylation sites. Consistent with this hypothesis, serum stimulation of S6K1 activity is associated with its incorporation into a calcium-dependent high-mol.-mass complex.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2004:187750 HCAPLUS

TITLE: Involvement of protein tyrosine phosphorylation and chloride channel in transient receptor potential protein mediated storeoperated calcium influx

AUTHOR(S): Qiu, Qinying; Yang, Xiaoru; He, Hua; Li, Jinliang; Wang, Xurong; Guan, Yongyuan

CORPORATE SOURCE: Department of Pharmacology, Zhongshan Medical College,

Sun Yat-Sen University, Guangzhou, 510089, Peop. Rep. China

SOURCE: Zhongguo Yaolixue Yu Dulixue Zazhi (2003), 17(3), 172-178
CODEN: ZYYZEW; ISSN: 1000-3002

PUBLISHER: Zhongguo Yaolixue Yu Dulixue Zazhi Biarjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB The possibility of involvement of protein tyrosine phosphorylation and Cl-channels in transient receptor potential protein mediated store-operated calcium influx (SOC) in HEK293 cells was analyzed. Human ThPL (hTRP1) and human TRP3 (hTRP3) cDNA were transfected to HEK293 cells resp. using lipofectAMINE reagent, the effect of genistein, furosemide and 4,4'-diisothiocyanostilbene-2.2-disulfonic acid (DIDS) on thapsigargin induced Ca2+ influx were determined by Fura-2/AM spectrophoto-fluorometry. H1EK293 cells was found, but not in hTRP3-transfected ones. Compared with untransfected cells the significant enhancement in TG-induced Ca2+ influx in hThPl - transfected HEK293 cells was found. Genistein 5 - 30 μmob L-1, fursemide 1 - 8 μmol L-1 and DIDS 0.5 - 1.0 μmol L-1 inhibited TG-induced Ca2+ influx in hThPl - transfected cells. HTRP1 protein might be one of components of TC-induced SOC in transfected HEK293 cells. Tyrosine phosphorylation and Cl-channels might be involved in TG-induced Ca2+ influx and tyrosine kinase might regulate hThPl protein directly.

L5 ANSWER 4 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:240979 HCAPLUS
DOCUMENT NUMBER: 136:274331
TITLE: Protein, gene and cDNA sequences of human calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof
INVENTOR(S): Beasley, Ellen M.; Wei, Ming-Hui; Bonazzi, Vivien R.; Sanders, Robert; Di Francesco, Valentina
PATENT ASSIGNEE(S): PE Corporation (NY), USA
SOURCE: PCT Int. Appl., 89 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002024920	A2	20020328	WO 2001-US29161	20010919
WO 2002024920	A3	20030313		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002086391	A1	20020704	US 2000-729995	20001206
US 6426206	B2	20020730		
AU 2001092755	A5	20020402	AU 2001-92755	20010919
EP 1320613	A2	20030625	EP 2001-973147	20010919
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002123121	A1	20020905	US 2002-135689	20020501
US 6670162	B2	20031230		
US 2004086926	A1	20040506	US 2003-690617	20031023

PRIORITY APPLN. INFO.: US 2000-233493P P 20000919

US 2000-247031P P 20001113
US 2000-729995 A 20001206
WO 2001-US29161 W 20010919
US 2002-135689 A3 20020501

AB The invention provides protein, cDNA and genomic sequences for a novel human protein, which shares sequence homol. to a known kinase protein, and is related to the calcium/calmodulin-dependent protein kinase kinase subfamily. The gene is expressed in the eye (retinoblastomas) and brain. Forty three novel single nucleotide polymorphism sites (beyond the ORF or in intron regions) were identified. Thus, the present invention specifically provides isolated peptide and nucleic acid mols., methods of identifying orthologs and paralogs of the calcium/calmodulin-dependent protein kinase kinase peptides, methods of identifying modulators of the calcium/calmodulin-dependent protein kinase kinase peptides, and methods of diagnosis and treatment of diseases associated with the calcium/calmodulin-dependent protein kinase kinase.

L5 ANSWER 5 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:127283 HCAPLUS
DOCUMENT NUMBER: 136:395825
TITLE: Nicotine activates the extracellular signal-regulated kinase 1/2 via the α7 nicotinic acetylcholine receptor and protein kinase A, in SH-SY5Y cells and hippocampal neurones
AUTHOR(S): Dajas-Bailador, F. A.; Soliakov, L.; Wonnacott, S.
CORPORATE SOURCE: Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, UK
SOURCE: Journal of Neurochemistry (2002), 80(3), 520-530
CODEN: JONRA9; ISSN: 0022-3042
PUBLISHER: Blackwell Publishing Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Neuronal nicotinic acetylcholine receptors (nAChR) can modulate many cellular mechanisms, such as cell survival and memory processing, which are also influenced by the serine/threonine protein kinases ERK1/2. In SH-SY5Y cells and hippocampal neurons, nicotine (100 μM) increased the activity of ERK1/2. This effect was Ca2+ dependent, and prevented by the α7 nAChR antagonist α-bungarotoxin (α-Bgt) and an inhibitor (PD98059) of the upstream kinase MEK. To determine the intervening steps linking Ca2+ entry to MEK-ERK1/2 activation, inhibitors of Ca2+-dependent kinases were deployed. In SH-SY5Y cells, selective blockers for PKC (Ro 31-8220), CaM kinase II (KN-62) or PI3 kinase (LY 294002) failed to inhibit the nicotine-evoked increase in ERK1/2 activity. In contrast, two structurally different inhibitors of PKA (KT 5720 and H-89) completely prevented the nicotine-dependent increase in ERK1/2 activity. Inhibition of the nicotine-evoked increase in ERK1/2 activity by H-89 was also observed in hippocampal cultures. Down stream of PKA, the activity of B-Raf was significantly decreased by nicotine in SH-SY5Y cells, as determined by direct measurement of MEK1 phosphorylation or in vitro kinase assays, whereas the modulation of MEK1 phosphorylation by Raf-1 tended to increase. Thus, this study provides evidence for a novel signaling route coupling the stimulation of α7 nAChR to the activation of ERK1/2, in a Ca2+ and PKA dependent manner.

REFERENCE COUNT: 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:853048 HCAPLUS
DOCUMENT NUMBER: 137:43065
TITLE: Kinase-dependent regulation of the intermediate conductance, calcium-dependent potassium channel, hIK1
AUTHOR(S): Gerlach, Aaron Charles
CORPORATE SOURCE: Univ. of Pittsburgh, Pittsburgh, PA, USA
SOURCE: (2000) 186 pp. Avail.: UMI, Order No. DA9998553

From: Diss. Abstr. Int., B 2001, 61(12), 6234
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L5 ANSWER 7 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:439719 BIOSIS
DOCUMENT NUMBER: PREV200000439719
TITLE: Determination of the genomic structure of calcium/calmodulin protein Kinase II gamma gene, a candidate gene for Type II diabetes.
AUTHOR(S): Gloyn, A. L. [Reprint author]; Hashim, Y. [Reprint author]; Ashcroft, S. J. H.
CORPORATE SOURCE: Diabetes Research Laboratories, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK
SOURCE: Diabetologia, (August, 2000) Vol. 43, No. Supplement 1, pp. A82. print.
Meeting Info.: 36th Annual Meeting of the European Association for the Study of Diabetes. Jerusalem, Israel. September 17-21, 2000. European Association for the Study of Diabetes.
CODEN: DBTGAJ. ISSN: 0012-186X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 18 Oct 2000
Last Updated on STN: 10 Jan 2002

L5 ANSWER 8 OF 28 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998198392 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9531494
TITLE: Activation of S6 **kinase** in **human** neutrophils by **calcium** pyrophosphate dihydrate crystals: protein kinase C-dependent and phosphatidylinositol-3-kinase-independent pathways.
AUTHOR: Tudan C; Jackson J K; Charlton L; Pelech S L; Sahl B; Burt H M
CORPORATE SOURCE: Department of Medicine, University of British Columbia, Biomedical Research Centre, Kinetek Biotechnology Corporation, Suite 500, 520 West 6th Avenue, Vancouver, BC, V5Z 1A1, Canada.
SOURCE: Biochemical journal, (1998 Apr 15) 331 (Pt 2) 531-7.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980716
Last Updated on STN: 19980716
Entered Medline: 19980706

AB Phosphatidylinositol 3-kinase (PI 3-kinase) has been shown previously to be a central enzyme in crystal-induced neutrophil activation. Since activation of the 70 kDa S6 kinase (p70S6K) has been shown to be dependent on PI 3-kinase activation in mammalian cells, and since the former is a key enzyme in the transmission of signals to the cell nucleus, activation of p70(S6K) was investigated in crystal-stimulated neutrophils. Cytosolic fractions from calcium pyrophosphate dihydrate (CPPD)-crystal-activated neutrophils were separated by Mono Q chromatography and analysed for phosphotransferase activity using a range of substrates and probed by Western analysis using antibodies to p70(S6K) and mitogen-activated protein kinase (MAP kinase). CPPD crystals induced a robust, transient activation (peak activity at 2 min) of p70(S6K) that was fully inhibited by pretreatment with rapamycin. This is the first report of the

activation of p70(S6K) in neutrophil signal transduction pathways induced by an agonist. This crystal-induced activation of p70(S6K) could also be inhibited by a protein kinase C (PKC) inhibitor (Compound 3), but not by the PI 3-kinase inhibitor wortmannin. CPPD crystals also activated the ERK1 and ERK2 forms of MAP kinase (wortmannin insensitive), PKC (Compound 3 sensitive) and protein kinase B (wortmannin sensitive) in neutrophils. These data suggest that activation of p70(S6K) may proceed through a PI 3-kinase- and protein kinase B-independent but PKC-dependent pathway in crystal-activated neutrophils.

L5 ANSWER 9 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:755955 HCAPLUS
DOCUMENT NUMBER: 123:221660
TITLE: Human calcium-calmodulin dependent protein kinase I: cDNA cloning, domain structure and activation by phosphorylation at threonine-177 by calcium-calmodulin dependent protein kinase I kinase
AUTHOR(S): Haribabu, Bodduluri; Hook, Sara S.; Selbert, Michele A.; Goldstein, Elaine G.; Tomhave, Eric D.; Edelman, Arthur M.; Snyderman, Ralph; Means, Anthony R.
CORPORATE SOURCE: Dep. Pharmacol., Duke Univ. Med. Cent., Durham, NC, 27710, USA
SOURCE: EMBO Journal (1995), 14(15), 3679-86
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human Ca2+-calmodulin (CaM) dependent protein kinase I (CaMKI) encodes a 370 amino acid protein with a calculated Mr of 41,337. The 1.5 kb CaMKI mRNA is expressed in many different human tissues and is the product of a single gene located on human chromosome 3. CaMKI 1-306, was unable to bind Ca2+-CaM and was completely inactive thereby defining an essential component of the CaM-binding domain to residues C-terminal to 306. CaMKI 1-294 did not bind CaM but was fully active in the absence of Ca2+-CaM, indicating that residues 295-306 are sufficient to maintain CaMKI in an auto-inhibited state. CaMKI was phosphorylated on Thr177 and its activity enhanced .apprx.25-fold by CaMKI kinase in a Ca2+-CaM dependent manner. Replacement of Thr177 with Ala or Asp prevented both phosphorylation and activation by CaMKI kinase and the latter replacement also led to partial activation in the absence of CaMKI kinase. Whereas CaMKI 1-306 was unresponsive to CaMKI kinase, the 1-294 mutant was phosphorylated and activated by CaMKI kinase in both the presence and absence of Ca2+-CaM although at a faster rate in its presence. These results indicate that the auto-inhibitory domain in CaMKI gates, in a Ca2+-CaM dependent fashion, accessibility of both substrates to the substrate binding cleft and CaMKI kinase to Thr177. Addnl., CaMKI kinase responds directly to Ca2+-CaM with increased activity.

L5 ANSWER 10 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:600410 HCAPLUS
DOCUMENT NUMBER: 119:200410
TITLE: ADP- and thapsigargin-evoked calcium entry and protein-tyrosine phosphorylation are inhibited by the tyrosine kinase inhibitors genistein and methyl-2,5-dihydroxycinnamate in fura-2-loaded human platelets
AUTHOR(S): Sargeant, Paul; Farndale, Richard W.; Sage, Stewart O.
CORPORATE SOURCE: Physiol. Lab., Univ. Cambridge, Cambridge, CB2 3EG, UK
SOURCE: Journal of Biological Chemistry (1993), 268(24), 18151-6
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors have investigated the mechanism of Ca2+ entry in fura-2-loaded

human platelets using the inhibitors of tyrosine kinases, genistein, and methyl-2,5-dihydroxycinnamate. Genistein (100 μM; 30 min) or methyl-2,5-dihydroxycinnamate (1 μg/mL; 30 min) reduced ADP-evoked protein-tyrosine phosphorylation at specific bands as assessed by gel electrophoresis and Western blotting with a specific antiphosphotyrosine antibody. Both compds. also reduced ADP-evoked [Ca2+]i rises in the presence, but not the absence, of external Ca2+, suggesting a relatively selective inhibition of Ca2+ entry over internal release. The inactive analog of genistein, daidzein, was without effect on protein-tyrosine phosphorylation or ADP-evoked Ca2+ elevation in the presence or absence of external Ca2+. Methyl-2,5-dihydroxycinnamate (1 μg/mL; 5 min) significantly reduced the Ca2+ influx evoked by depletion of the intracellular Ca2+ stores using the inhibitor of the endomembranous Ca2+-ATPase, thapsigargin. These results with tyrosine kinase inhibitors are unlikely to be the result of the inhibition of other protein kinases since kinases A, C, and G all inhibit agonist-evoked rises in [Ca2+]i in platelets. These data support a role for tyrosine kinases in the control of Ca2+ entry in human platelets.

L5 ANSWER 11 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

ACCESSION NUMBER: 1993:390096 BIOSIS
DOCUMENT NUMBER: PREV199396065396
TITLE: Synthetic peptides corresponding to the calmodulin-binding domain of skeletal muscle myosin light chain **kinase** and **human** erythrocyte **calcium** pump interact with and permeabilize liposomes and cell membranes.
AUTHOR(S): Eshel, Yoav; Shai, Yechiel; Vorherr, Thomas; Carafoli, Ernesto; Salomon, Yoram [Reprint author]
CORPORATE SOURCE: Dep. Hormone Res., Weizmann Inst. Sci., 76100 Rehovot, Israel
SOURCE: Biochemistry, (1993) Vol. 32, No. 26, pp. 6721-6728. CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Aug 1993
Last Updated on STN: 24 Aug 1993

AB Synthetic calmodulin-binding (CaM-binding) peptides (CBPs) representing CaM-binding domains of Ca-2+/CaM-dependent enzymes have been reported to interfere with the activity of the melanocyte-stimulating hormone (MSH) receptor function in melanoma cells (Gerst, J. E., and Salomon, Y. (1988) J. Biol. Chemical 263, 7073-7078). We postulated that membrane lipids may play an important role in the mode of action of CBPs on cells. We therefore tested the ability of CBPs to interact with membrane bilayers. Using artificial phospholipid vesicles, or M2R melanoma cells and cell membranes derived therefrom, as models, we report here that synthetic peptides representing the CaM-binding domains of skeletal muscle myosin light chain kinase (M5) and the human erythrocyte calcium pump (C28W), as well as other CBPs, interact with lipid bilayers and cell membranes. Significant interactions of CBPs with the lipid bilayer were detected in both model systems. M5 and C28W were found to partition into the lipid bilayer of melanoma cell membranes and soybean lecithin vesicles, and surface partition constants obtained (for the liposome model) were in the range 10-3-10-4 M-1. In addition, C28W and its N-modified NBD derivative were found to inhibit (125I)iodo-(Nle-4,D-Phe-7)alpha-MSH binding to cultured M2R melanoma cells. These and other CBPs were also found to induce the release of cations and calcein from liposomes, suggesting that the interaction of CBPs with the lipid bilayer increases membrane permeability. Nonrelevant peptides used as controls were found ineffective. Melittin, a bee venom derived CBP, and pardaxin, a shark-repellent neurotoxin, both membrane-permeating peptides, were in comparison more potent than the enzyme-derived CBPs that were not lytic when applied to cells. It is proposed that the tested CBPs act as

permeators that partition into the lipid bilayer of the cell membrane, thereby also promoting their interaction with hydrophobic domains of membrane proteins such as the MSH receptor, consequently eliciting the observed cellular responses.

L5 ANSWER 12 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:403901 HCAPLUS
DOCUMENT NUMBER: 119:3901
TITLE: Cloning and analysis of two new isoforms of multifunctional calcium/calmodulin-dependent protein kinase. Expression in multiple human tissues
AUTHOR(S): Nghiem, Paul; Saati, Shahin M.; Martens, Christine L.; Gardner, Phyllis; Schulman, Howard
CORPORATE SOURCE: Dep. Pharmacol., Stanford Med. Sch., Stanford, CA, 94305, USA
SOURCE: Journal of Biological Chemistry (1993), 268(8), 5471-9
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Multifunctional Ca2+/calmodulin-dependent protein kinase (CaM kinase) is a mediator of calcium signals in diverse signaling pathways. In human lymphocytes and epithelial tissues, CaM kinase activates a chloride channel via a Ca2+-dependent pathway which is preserved in cystic fibrosis. To characterize the CaM kinase present in these tissues the authors have cloned an isoform of this kinase from human T lymphocytes. The cDNA sequences of 2 variants of this human CaM kinase, γB and γC, are predicted to translate to 518 and 495 amino acids, resp. Amino acid differences between these isoforms and the rat brain γ isoform (which the authors refer to as γA) are localized to the variable domain. RNase protection of this variable region revealed the level of expression of γB and γC CaM kinase mRNAs in nine human tissues and cell lines. When transfected into Jurkat T cells, the γB cDNA encoded a functional kinase which cosedimented on sucrose gradients with endogenous T cell CaM kinase activity and formed a large multimeric enzyme. The recombinant γB isoform displayed 2 phases of autophosphorylation characteristic of CaM kinases, including the phase which converts it to a partially Ca2+-independent species. Site-directed mutagenesis of the predicted autoinhibitory domain yielded a mutant which was .apprx.37% active in the absence of Ca2+/calmodulin, confirming the region as critical for autoregulation, and suggesting this mutant as a tool for studying the role of CaM kinase in nonneuronal tissues.

L5 ANSWER 13 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:79261 HCAPLUS
DOCUMENT NUMBER: 118:79261
TITLE: Rapid priming of calcium mobilization and superoxide anion production in human neutrophils by substimulatory concentrations of phorbol esters: a novel role for protein kinase C and tyrosine phosphorylation in the up-modulation of signal transduction
AUTHOR(S): Gilbert, Caroline; Gaudry, Murielle; Naccache, Paul H.
CORPORATE SOURCE: Cent. Rech. Inflammation, Immunol. Rhumatol., CHUL, Ste-Foy, QC, G1V 4G2, Can.
SOURCE: Cellular Signalling (1992), 4(5), 511-23
CODEN: CESIEY; ISSN: 0898-6568
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The modulatory influences of phorbol esters on the functional responsiveness of human peripheral blood neutrophils have been investigated. These studies focused on measurements of the levels of cytoplasmic free calcium and of tyrosine phosphorylation as well as on their ability to mount an oxidative response. Short incubation times (<1 min) with low concns. of phorbol esters (5-50 nM) were shown to enhance

the above indexes of neutrophil responsiveness to chemotactic factors such as fMet-Leu-Phe and leukotriene B4. On the other hand, a time- and concentration-dependent inhibition of calcium mobilization and superoxide production
was also observed The effects of the phorbol esters were stereospecific and were antagonized by a novel protein kinase C inhibitor (RO 318220) but were not affected by the oxidative burst inhibitor diphenyleneiodonium. Pre-incubation of the cells with phorbol 12,13-dibutyrate (PDBu) altered in a concentration-dependent manner the tyrosine phosphorylation pattern stimulated by fMet-Leu-Phe. In addition, the tyrosine kinase inhibitor erbstatin inhibited the priming of the mobilization of calcium induced by PDBu. These data demonstrate the rapidity of the effects of the activation of protein kinase C, their potential to modulate pos. the early events of the excitation-response coupling sequence and the complexity of the functional interrelationships among the various cellular activation pathways available to human neutrophils and other nonmuscle cells.

L5 ANSWER 14 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1991:466962 BIOSIS
DOCUMENT NUMBER: PREV199141092722; BR41:92722
TITLE: CALCIUM-CALMODULIN DEPENDENT ACTIVATION OF GLUCOCORTICOID RECEPTORS IN ALOPECIA AREATA.
AUTHOR(S): SAWAYA M E [Reprint author]; HORDINSKY M K; SCHMIEDER G J
CORPORATE SOURCE: UNIV MIAMI SCH MED, MIAMI, FLA, USA
SOURCE: Clinical Research, (1991) Vol. 39, No. 2, pp. 474A.
Meeting Info.: MEETING OF THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, SEATTLE, WASHINGTON, USA, MAY 1-4, 1991. CLIN RES.
CODEN: CLREAS. ISSN: 0009-9279.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 21 Oct 1991
Last Updated on STN: 21 Oct 1991

L5 ANSWER 15 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1991:182802 HCAPLUS
DOCUMENT NUMBER: 114:182802
TITLE: Signal transduction and the regulation of actin conformation during myeloid maturation: studies in HL60 cells
AUTHOR(S): Sham, Ronald L.; Packman, Charles H.; Abboud, Camille N.; Lichtman, Marschall A.
CORPORATE SOURCE: Med. Cent., Univ. Rochester, Rochester, NY, USA
SOURCE: Blood (1991), 77(2), 363-70
CODEN: BLOOAW; ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Maturation of human myeloid cells is associated with quant. and qual. changes in protein kinase C (PKC) and increases in N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) receptors, actin, and actin regulatory proteins. The actin responses and cell shape changes caused by FMLP and its 2nd messenger pathways in HL60 cells undergoing neutrophilic maturation were studied. In uninduced cells, the PKC activators TPA, bryostatin, and 1-oleyl-2-acetylglycerol (OAG) resulted in 15-30% decreases in F-actin, whereas FMLP had no effect. Ionomycin had no effect on actin but did cause a 10-fold increase in intracellular Ca. Cells grown for 24 h in 1% DMSO acquired the ability to polymerize actin in response to FMLP and ionomycin. TPA continued to cause a decrease in F-actin at 24 h, but caused an increase in F-actin at 48-72 h of maturation. The PKC inhibitor 1-5-isoquinolinesulfonyl 2-methylpiperazine (H7) partially blocked the F-actin increase caused by TPA in induced cells, but had no effect on the decrease in F-actin caused by TPA in uninduced cells or the increase in F-actin seen in FMLP-treated neutrophils. F-actin rich pseudopods

developed following TPA or FMLP stimulation of induced HL60 cells; in uninduced cells neither agent caused pseudopod formation but TPA caused a dramatic loss of surface ruffles. The ability of FMLP and ionomycin to elicit a neutrophil-like actin response in HL60 cells within 24 h after DMSO treatment shows that the actin regulatory mechanism is mature by that time. The inability of ionomycin to increase F-actin in uninduced cells supports the view that Ca increases alone are insufficient for actin polymerization The longer maturation time required for HL60 cells to develop an actin polymerization response to TPA compared with FMLP, coupled with the inability of H7 to block the FMLP-mediated F-actin increase in neutrophils, suggests that the F-actin increase caused by FMLP is not mediated solely by PKC. Lastly, the TPA-induced F-actin decrease and shape changes in uninduced HL60 cells, and the longer time required for a mature response to TPA, may reflect immaturity in the PKC isoenzyme pattern rather than immaturity of the actin regulatory mechanism.

L5 ANSWER 16 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:457273 HCAPLUS
DOCUMENT NUMBER: 113:57273
TITLE: Translocation of protein kinase C in human polymorphonuclear neutrophils. Regulation by cytosolic calcium-independent and calcium-dependent mechanisms
AUTHOR(S): O'Flaherty, Joseph T.; Jacobson, David P.; Redman, Jimmy F.; Rossi, Adriano G.
CORPORATE SOURCE: Med. Cent., Wake Forest Univ., Winston-Salem, NC, 27013, USA
SOURCE: Journal of Biological Chemistry (1990), 265(16), 9146-52
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB [3H]Phorbol dibutyrate ([3H]PDB) rapidly and reversibly binds to human polymorphonuclear neutrophils (PMN). Ca2+/diacylglycerol/phospholipid-dependent protein kinase C appeared to be the receptor for this binding because; a diacylglycerol, dioctanoylglycerol, competed with [3H]PDB for PMN binding sites; a blocker of protein kinase C-phospholipid interactions, sphinganine, inhibited PMN binding of [3H]PDB; and changes in cytosolic Ca2+ apparently regulated PMN binding of the label. Results from Ca ionophore studies fit the standard model, wherein rises in cytosolic Ca2+ cause protein kinase C to translocate from cytosol to plasmalemma and thereby become more available to [3H]PDB. In contrast, two humoral agonists, N-formyl-Met-Leu-Phe (fMLP) and leukotriene (LT)B4, had actions that did not fit this model. They stimulated PMN to increase the availability of PDB binding sites by a sphinganine-sensitive mechanism but their actions differed from those of ionophores. They induced biphasic increases in [3H]PDB binding while eliciting monophasic short-lived ($t < 1$ min) rises in cytosolic Ca2+. In Ca2+-depleted PMN, fMLP and LTB4 stimulated slow monophasic, prominent rises in [3H]PDB binding and binding site number without appreciably altering cytosolic Ca2+. It is suggested that fMLP and LTB4 translocate protein kinase C using two sequential mechanisms. The first involves Ca2+ transients and thus produces abrupt ($t = 15$ s), rapidly reversing responses. The second mechanism uses an unrelated signal to effect a more slowly evolving ($t = 60$ s) movement of protein kinase C to plasmalemma. Hence, the standard model does not explain all instances of protein kinase C translocation, and a cytosolic Ca2+-independent signal contributes to the regulation of protein kinase C as well as those responses elicited by the effector enzyme.

L5 ANSWER 17 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:3362 HCAPLUS
DOCUMENT NUMBER: 112:3362
TITLE: Tyrosine-specific phosphorylation of calmodulin by the

insulin receptor kinase purified from human placenta

AUTHOR(S): Sacks, David B.; Fujita-Yamaguchi, Yoko; Gale, R. Dane; McDonald, Jay M.

CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA

SOURCE: Biochemical Journal (1989), 263(3), 803-12
CODEN: BIJOAK; ISSN: 0306-3275

DOCUMENT TYPE: Journal

LANGUAGE: English

AB It has previously been demonstrated that calmodulin can be phosphorylated in vitro and in vivo by both tyrosine-specific and serine/threonine protein kinase. Here it is demonstrated that the insulin receptor tyrosine kinase purified from human placenta phosphorylates calmodulin. The highly purified receptors (prepared by insulin-Sepharose chromatog.) were 5-10-times more effective in catalyzing the phosphorylation of calmodulin than an equal number of partially purified receptors (prepared by wheat germ agglutinin-Sepharose chromatog.). Phosphorylation occurred exclusively on tyrosine residues, up to a maximum of 1 mol of phosphate incorporated/mol of calmodulin. Phosphorylation of calmodulin was dependent on the presence of certain basic proteins and divalent cations. Some of these basic proteins, i.e. polylysine, polyarginine, polyornithine, protamine sulfate, and histones H1 and H2B, were also able to stimulate the phosphorylation of calmodulin via an insulin-independent activation of the receptor tyrosine kinase. Addition of insulin further increased incorporation of 32P into calmodulin. The magnitude of the effect of insulin was dependent on the concentration and type of basic protein used, ranging from 0.5- to 9.0-fold stimulation. Maximal phosphorylation of calmodulin was obtained at an insulin concentration of 9.0-fold stimulation. Maximal phosphorylation of calmodulin was obtained at an insulin concentration of

10-10M, with half-maximal effect at 10-11M. Either Mg2+ or Mn2+ was necessary to obtain phosphorylation, but Mg2+ was far more effective than Mn2+. In contrast, maximal phosphorylation of calmodulin was observed in the absence of Ca2+. Inhibition of phosphorylation was observed as free Ca2+ concentration exceeded 0.1 μM, with almost complete inhibition at 30 μM free Ca2+. The Km for calmodulin was .apprx.0.1 μM. To gain further insight into the effects of basic proteins in this system, the binding of calmodulin to the insulin receptor and the polylysine was examined Calmodulin binds to the insulin receptor in a Ca2+-dependent manner, whereas it binds to polylysine seemingly by electrostatic interactions. These studies identify calmodulin as a substrate for the highly purified insulin receptor tyrosine kinase of human placenta. They also demonstrate that the basic proteins, which are required for insulin to stimulate the phosphorylation of calmodulin, do so by a direct interaction with calmodulin.

L5 ANSWER 18 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1988:218057 HCAPLUS

DOCUMENT NUMBER: 108:218057

TITLE: Characterization of phosphatidylinositol and phosphatidylinositol-4-phosphate kinases in human neutrophils

AUTHOR(S): Pike, Marilyn C.; Arndt, Christina

CORPORATE SOURCE: Med. Cent., Univ. Michigan, Ann Arbor, MI, 48109, USA

SOURCE: Journal of Immunology (1988), 140(6), 1967-73
CODEN: JOIMA3; ISSN: 0022-1767

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Phosphodiesteric cleavage of phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P2) is required for transmembrane signaling by chemoattractants in human polymorphonuclear leukocytes (PMN). Because of the importance of PtdIns-4,5-P2 as a reservoir for 2nd messenger substances, the enzyme system that synthesizes this phospholipid in human PMN was characterized. The system consists of kinases for phosphatidylinositol (PtdIns) and phosphatidylinositol-4-phosphate

(PtdIns-4-P). ATP was preferred over GTP as phosphate donor for both enzymes. The resp. Km for ATP for PtdIns kinase and PtdIns-4-P kinase were 0.049 and 0.062 mM and for GTP were 0.242 and 0.186 mM. PtdIns stimulated PtdIns kinase to a greater extent than PtdIns-4-P kinase. PtdIns-4-P inhibited detergent-solubilized PtdIns kinase and stimulated particulate PtdIns-4-P kinase, whereas both enzymes exhibited substrate inhibition by PtdIns-4,5-P2. Mg2+ was the preferred cation for both enzymes, but the apparent Km values (4.1 mM for PtdIns kinase and 1.0 mM for PtdIns-4-P kinase) differed significantly. Mn2+ partially substituted for Mg2+, and both enzymes were inhibited by Ca2+. The polyamine spermine stimulated PtdIns-4-P kinase activity to a greater extent and at lower concns. than PtdIns kinase. PtdIns kinase was easily solubilized in both Triton X-100 and Nonidet P-40, whereas PtdIns-4-P kinase remained in a detergent-nonextractable membrane fraction. Thus, the enzyme system in human PMN that forms PtdIns-4,5-P2 is composed of 2 distinct enzymes with similar characteristics.

L5 ANSWER 19 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3

ACCESSION NUMBER: 1989:747 BIOSIS
DOCUMENT NUMBER: PREV198987000747; BA87:747
TITLE: CALCIUM MOBILIZATION PRIMES PROTEIN **KINASE** C IN **HUMAN** PLATELETS **CALCIUM** AND PHORBOL ESTERS STIMULATE PLATELET AGGREGATION AND SECRETION SYNERGISTICALLY THROUGH PROTEIN KINASE C.
AUTHOR(S): SIESS W [Reprint author]; LAPETINA E G
CORPORATE SOURCE: MOL BIOL DEP, BURROUGHS WELLCOME CO, RESEARCH TRIANGLE PARK, NC 27709, USA
SOURCE: Biochemical Journal, (1988) Vol. 255, No. 1, pp. 309-318. ISSN: 0264-6021.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 6 Dec 1988
Last Updated on STN: 6 Dec 1988

AB Low concentrations of Ca2+-mobilizing agonists such as vasopressin, platelet-activating factor, ADP, the endoperoxide analogue U44069 and the Ca2+ ionophore A23187 enhance the binding of [3H]phorbol 12,13-dibutyrate (PdBu) to intact human platelets. This effect is prevented by preincubation of platelets with prostacyclin (except for A23187). Adrenaline, which does not increase Ca2+ in the platelt cytosol, does not enhance the binding of [3H]PdBu to platelets. In addition, all platelet agonsits except adrenaline potentiate the phosphorylation of the substrate of protein kinase C (40 kDa protein) induced by PdBu. Potentiation of protein kinase C activation is associated with increased platelet aggregation and secretion. Stimulus-induced myosin light-chain phosphorylation and shape change are not significantly affected, but formation of phosphatidic acid is decreased in the presence of PdBu. The results may indicate that low concentrations of agonists induce in intact platelets the translocation of protein kinase C to the plasma membrane by eliciting mobilization of Ca2+, and thereby place the enzyme in a strategic position for activation by phorbol ester. Such activation enhances platelet aggregation and secretion, but at the same time suppresses activation of phospholipase C. Therefore, at least part of the synergism evoked by Ca2+ and phorbol ester is mediated through a single pathway which involves protein kianse C. It is likely that the priming of protein kinase C by prior Ca2+ mobilization occurs physiologically in activated platelets.

L5 ANSWER 20 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:506943 HCAPLUS
DOCUMENT NUMBER: 109:106943
TITLE: Human neutrophil protein kinase C: calcium-induced changes in the solubility of the enzyme do not always

correlate with enzymic activity
AUTHOR(S): Balazovich, Kenneth J.; Boxer, Laurence A.
CORPORATE SOURCE: Dep. Pediatr., Univ. Michigan, Ann Arbor, MI, 48109, USA
SOURCE: Biochimica et Biophysica Acta (1988), 970(3), 305-17
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Ca2+ and 1,2-diacylglycerols were hypothesized to stimulate human neutrophil (PMN) protein kinase C (EC 2.7.1.37) in a 2-step mechanism. The proposed mechanism entails (1) increased insol. protein kinase C activity and (2) endogenous protein phosphorylation, events which have not been biochem. dissociated PMN which were treated with 100 nM ionomycin shifted protein kinase C activity from being mostly soluble to insol. Concns. of ionomycin of >300 nM stimulated a doubling of total cellular (soluble + insol.) protein kinase activity and stimulated increased endogenous phosphorylation of PMN proteins. Intracellular Ca2+ (measured with fura-2) increased from 65 nM (basal) to 680 nM using 500 nM ionomycin; Ca2+ increases were dose-dependent. The anti-inflammatory agents, acetylsalicylate and Na salicylate (but not ibuprophen, indomethacin, or acetaminophen), inhibited ionomycin-induced protein kinase C activation and protein phosphorylation in a dose-dependent manner by inhibiting the production of diacylglycerols. 1-Oleoyl-2-acetylglycerol reversed the inhibitory effect of salicylates. In contrast to the effect of acetylsalicylates on protein kinase C functional activity, the distribution of phorbol receptors was unaffected in acetylsalicylate-treated, ionomycin-stimulated PMN using a phorbol-binding assay. The results showed that ionomycin increased intracellular diacylglycerol levels 3.5-fold over those present in control PMN, whereas acetylsalicylate decreased diacylglycerol production in ionomycin-stimulated PMN below baseline values. These results support the hypothesis that increased intracellular Ca2+-activated protein kinase C leading to protein phosphorylation in 2 distinct dissociable events: (1) increased intracellular Ca2+; and (2) increased 1,2-diacylglycerol levels.

L5 ANSWER 21 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:546974 HCAPLUS
DOCUMENT NUMBER: 109:146974
TITLE: The regulatory roles of inositol trisphosphate, cyclic AMP-dependent and calmodulin-dependent protein **kinases** in **human** platelet **calcium** homeostasis
AUTHOR(S): Adunyah, Samuel Evans
CORPORATE SOURCE: Univ. Louisville, Louisville, KY, USA
SOURCE: (1987) 183 pp. Avail.: Univ. Microfilms Int., Order No. DA8801959
From: Diss. Abstr. Int. B 1988, 48(11), 3270
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L5 ANSWER 22 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1987:98408 HCAPLUS
DOCUMENT NUMBER: 106:98408
TITLE: Calcium-calmodulin-dependent protein kinase in hyperplastic human parathyroid glands
AUTHOR(S): Kinder, B. K.; Delahunt, N. G.; Jamieson, J. D.; Gorelick, F. S.
CORPORATE SOURCE: Sch. Med., Yale Univ., New Haven, CT, 06510, USA
SOURCE: Endocrinology (1987), 120(1), 170-7
CODEN: ENDOAO; ISSN: 0013-7227
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hyperplastic human parathyroid tissue was examined for Ca2+ and calmodulin

(CaM)-dependent protein kinase activity. In parathyroid homogenates, Ca2+ stimulates the phosphorylation of substrate protein in the presence of calmodulin or phospholipid. The CaM-stimulated activity is present in a soluble fraction of parathyroid and can be separated from other protein kinase activities by gel filtration chromatog. The concentration dependence of CaM kinase on Ca2+ and CaM was determined using the gel filtration fraction. The Ka values for CaM and Ca were 100 nM and 5 μM, resp. The fraction containing the CaM kinase activity has a calculated mol. weight of 5.5 + 105. It contained a protein with a mol. weight of 4.9 + 104 whose phosphorylation was Ca2+ CaM dependent and a CaM-binding protein of mol. weight 4.9 + 104 which may be the catalytic subunit of a type II Ca2+-CaM dependent protein kinase. Hyperplastic human parathyroid tissue contains a type II Ca2+-CaM dependent protein kinase which may serve an important function in Ca2+-directed metabolism

L5 ANSWER 23 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1987:193468 HCAPLUS
DOCUMENT NUMBER: 106:193468
TITLE: Synergistic effect of retinoic acid and calcium ionophore A23187 on differentiation, c-myc expression, and membrane tyrosine kinase activity in human promyelocytic leukemia cell line HL-60
AUTHOR(S): Chapekar, Mrunal S.; Hartman, Kathleen D.; Knode, Marian C.; Glazer, Robert I.
CORPORATE SOURCE: Lab. Biol. Chem., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SOURCE: Molecular Pharmacology (1987), 31(2), 140-5
CODEN: MOPMA3; ISSN: 0026-895X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effect of the combination of retinoic acid (RA) and the Ca2+ ionophore A 23187 on cellular differentiation was assessed in the promyelocytic leukemia cell line HL-60. RA (10-10-2.5 + 10-8M) or A 23187 (4 + 10-7M) alone produced 15-22% differentiated cells as assessed by nitroblue tetrazolium reduction Exposure of cells for 48 h to the combination of 4 + 10-7M A 23187 and 10-10-2.5 + 10-8M RA resulted in 20-86% of the cells capable of reducing nitroblue tetrazolium, but with no measurable level of nonspecific esterase activity. The combination of A 23187 and either DMSO, 1,25-dihydroxyvitamin D3, or immune interferon failed to produce a synergistic effect on differentiation. Addition of either the calmodulin antagonists N-(6-aminohexyl)-5-chloronaphthalenesulfonamide and trifluoperazine or the protein kinase C inhibitor 1-(5-isoquinolinesulfonyl)-2-methylpiperazine during treatment with A 23187 and RA did not block differentiation. Membrane tyrosine kinase activity was measured in cells treated with A 23187 and RA in a nondenaturing gel system by using the exogenous substrate poly(Glu:Tyr). Membrane-bound tyrosine kinase activity was not present in untreated or RA-treated cells but was induced by A 23187 treatment alone and was markedly increased in cells 48 h after treatment with the combination of A 23187 and RA. Significantly greater reduction in c-myc mRNA levels was also observed 24 h after treatment with RA and A 23187 in comparison to that observed with either agent alone. Thus, a Ca2+-mediated process sensitizes cells to the differentiating effect of RA, and this effect is associated with a significant reduction of c-myc expression and the induction of membrane tyrosine kinase activity in this cell line.

L5 ANSWER 24 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:619353 HCAPLUS
DOCUMENT NUMBER: 105:219353
TITLE: Stimulation of creatine kinase activity by calcium-regulating hormones in explants of human amnion, decidua, and placenta
AUTHOR(S): Weisman, Y.; Golander, A.; Spirer, I. Binderman Z.;

Kaye, A. M.; Somjen, D.
CORPORATE SOURCE: Sackler Sch. Med., Tel Aviv Univ., Tel Aviv, 64239, Israel
SOURCE: Journal of Clinical Endocrinology and Metabolism (1986), 63(5), 1052-6
CODEN: JCEMAZ; ISSN: 0021-972X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Stimulation of the brain type creatine kinase (CK) [9001-15-4] isoenzyme was used as a response marker to examine the effects of vitamin D metabolites, parathyroid hormone (PTH) [9002-64-6], and bovine calcitonin [26112-29-8] in cultured explants of placenta, decidua, and amnion from normal human deliveries. There was a biol. response to PTH in placenta and amnion and to vitamin D metabolites in all 3 tissues. In the amnion, CK activity increased 2.3-fold after 24 h of incubation in 2.5 nM 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] [32511-63-0], 3.8-fold when incubated with 12.5 nM 24,25-dihydroxyvitamin D3 [24,25-(OH)2D3] [40013-87-4], and 2.7-fold when incubated with 10 units bovine PTH/mL.. In the decidua, 24,25-(OH)2D3, but not 1,25-(OH)2D3 or bPTH, caused a 1.7-fold increase in CK activity. In contrast, the placenta responded to 1,25-(OH)2D3 with a 1.6-fold increase in CK activity and to bPTH with a 1.7-fold increase, but did not respond to 24,25-(OH)2D3. Bovine calcitonin (100 ng/mL) had no effect on CK activity in any of the 3 tissues. Nearly all CK in both the unstimulated and stimulated explants was the brain type isoenzyme. CK activity increased 1-4 h after hormonal treatment in all expts. The enzyme activity rose steeply with dose and reached an increase, and usually a plateau, at hormone concns. considered to be physiol. in vivo. [3H]Thymidine incorporation into DNA increased in parallel to stimulation of CK activity in all expts., except that PTH did not increase DNA synthesis in the placenta. PTH did cause an increase in cAMP [60-92-4] production in explants of amnion (1.5-fold) and placenta (2.6-fold).

L5 ANSWER 25 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:494958 HCAPLUS
DOCUMENT NUMBER: 105:94958
TITLE: Protein kinase C in human brain and its inhibition by calmodulin
AUTHOR(S): Saitoh, Tsunao; Dobkins, Karen R.
CORPORATE SOURCE: Sch. Med., Univ. California, San Diego, La Jolla, CA, 92093, USA
SOURCE: Brain Research (1986), 379(1), 196-9
CODEN: BRREAP; ISSN: 0006-8993
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein kinase C is a unbiquitous enzyme which is especially concentrated in brain
tissue and which has been reported to have a key role in the regulation of neuronal activity. Substrates for this kinase were studied in frozen postmortem human cortex. Two major protein substrates with mol. wts. of 86,000 and 67,000 were found in the cytosol fraction. Calmodulin inhibited phosphorylation of these 2 proteins.

L5 ANSWER 26 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:438087 HCAPLUS
DOCUMENT NUMBER: 105:38087
TITLE: Calcium and phorbol ester activation of protein kinase C at intracellular calcium concentrations and the effect of TMB-8
AUTHOR(S): Christiansen, Niels Obel; Larsen, Carsten Schade; Juhl, Henning
CORPORATE SOURCE: Dep. Med. Infect. Dis., Marselisborg Hosp., Aarhus, DK-8000, Den.
SOURCE: Biochimica et Biophysica Acta (1986), 882(1), 57-62

CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A Ca-activated, phospholipid-dependent protein kinase (protein kinase C) was purified to near homogeneity from human polymorphonuclear leukocytes and shown to be identical to bovine protein kinase C. The Ca2+ activation of the enzyme was studied and the Ca2+ concns. required to activate the enzyme were compared to free cytosolic Ca2+ concns. in resting and activated polymorphonuclear leukocytes. The free Ca2+ concns. in the cytosol and in the enzyme assay mixture were determined by using the Ca indicator quin 2. The enzyme activity was almost totally dependent upon phosphatidylserine and could be strongly activated by Ca2+ concns. in the micromolar range, but was not activated by phosphatidylserine at Ca2+ concns. corresponding to the intracellular free Ca2+ concentration under resting conditions. However, at similar Ca2+ concns. (<2.5·10-7M), the enzyme was highly activated by phorbol 12-myristate 13-acetate (PMA) or diolein in the presence of phosphatidylserine. PMA stimulation of human polymorphonuclear leukocytes did not induce any increase in the level of the intracellular free Ca concentration PMA activation of protein kinase C occurred independently of a rise in the intracellular Ca2+ concentration K0.5 (Half-maximal activation) for the PMA activation of purified protein kinase C was equivalent to the K0.5 for PMA stimulation of O2 production in human polymorphonuclear leukocytes, suggesting that protein kinase C is involved in activation of the NADPH oxidase. The presumed intracellular Ca2+ antagonist TMB 8 inhibited the PMA-induced O2- production, but neither by an intracellular Ca2+ antagonism nor by a direct inhibition of protein kinase C activity.

L5 ANSWER 27 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1984:524592 HCAPLUS
DOCUMENT NUMBER: 101:124592
TITLE: Modulation of calcium-activated, phospholipid-dependent protein kinase in platelets treated with a tumor-promoting phorbol ester
AUTHOR(S): Tapley, Peter M.; Murray, Andrew W.
CORPORATE SOURCE: Sch. Biol. Sci., Flinders Univ. South Australia, Bedford Park, 5042, Australia
SOURCE: Biochemical and Biophysical Research Communications (1984), 122(1), 158-64
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Incubation of human platelets with TPA (I) [16561-29-8] caused a rapid decrease in soluble Ca2+, phospholipid-dependent protein kinase [9026-43-1] activity (protein kinase C) and an increase in protein kinase C associated with the particulate fraction. TPA also induced an increased activity of a Ca2+, phospholipid-independent protein kinase activity in the soluble and particulate fractions of the platelets. This latter kinase eluted from DEAE cellulose columns at a higher salt concentrate than protein kinase C, and was shown by Sephadex G-100 chromatog. to have a mol. weight of .apprx.50,000 compared with a mol. weight of 80,000 for protein kinase C. Thus, TPA treatment of platelets causes irreversible activation of protein kinase C by proteolysis of the enzyme to a form active in the absence of Ca2+ and phospholipid.

L5 ANSWER 28 OF 28 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1982:99951 HCAPLUS
DOCUMENT NUMBER: 96:99951
TITLE: Calmodulin and acidic compounds alter basic protein phosphorylation by the protein kinase from human platelets

AUTHOR(S): Yamaki, Tokuo; Nishikawa, Masakatsu; Hidaka, Hiroyoshi
CORPORATE SOURCE: Sch. Med., Mie Univ., Tsu, 514, Japan
SOURCE: Biochimica et Biophysica Acta (1982), 714(2), 257-64
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A cyclic nucleotide-independent protein kinase (I) of human blood platelets, which phosphorylated histones, myelin basic protein, and protamine, and did not catalyze the phosphorylation of acidic proteins, such as casein, phosvitin, and myosin light chain, was purified .apprx.1500-fold from the crude extract by DEAE-cellulose, Sephadex G-200, hydroxylapatite, and phosphorylcellulose column chromatog. The substrate phosphorylation by I was markedly enhanced by calmodulin even in the absence of Ca2+, when mixed histone was used as a substrate. The interaction of I with mixed histone resulted in an irreversible inactivation of the enzyme. Calmodulin prevented this inactivation and produced an apparent increase in histone phosphorylation by I. Acidic polypeptides, such as troponin C, phospholipids, and nucleic acids have a similar ability. The addition of Ca2+ reduced the effect of calmodulin more than the effects of other acidic compds.

=> d his

(FILE 'HOME' ENTERED AT 13:24:46 ON 30 JUL 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 13:25:29 ON 30 JUL 2004
L1 1222343 S KINASE?
L2 2111610 S CALCIUM OR CALMODULIN
L3 21392 S L1(4W)L2
L4 34 S HUMAN (A)L3
L5 28 DUP REM L4 (6 DUPLICATES REMOVED)

=> s clon? or express? or recombinant
5 FILES SEARCHED...
L6 6635055 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l6
L7 5864 L3 AND L6

=> s human and l7
L8 1850 HUMAN AND L7

=> s retinoblastomas
L9 1641 RETINOBLASTOMAS

=> s l8 and l9
L10 1 L8 AND L9

=> d all

L10 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2002:240979 HCAPLUS
DN 136:274331
ED Entered STN: 28 Mar 2002
TI Protein, gene and cDNA sequences of **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof
IN Beasley, Ellen M.; Wei, Ming-Hui; Bonazzi, Vivien R.; Sanders, Robert; Di Francesco, Valentina
PA PE Corporation (NY), USA
SO PCT Int. Appl., 89 pp.
CODEN: PIXXD2

DT Patent
LA English
IC ICM C12N015-54
ICS C12N009-12; C07K016-40; C12Q001-68; A01K067-027; C12N005-10; G01N033-53
CC 3-3 (Biochemical Genetics)
Section cross-reference(s): 1, 6, 13
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002024920	A2	20020328	WO 2001-US29161	20010919
	WO 2002024920	A3	20030313		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	US 2002086391	A1	20020704	US 2000-729995	20001206
	US 6426206	B2	20020730		
	AU 2001092755	A5	20020402	AU 2001-92755	20010919
	EP 1320613	A2	20030625	EP 2001-973147	20010919
	R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	US 2002123121	A1	20020905	US 2002-135689	20020501
	US 6670162	B2	20031230		
	US 2004086926	A1	20040506	US 2003-690617	20031023
PRAI	US 2000-233493P	P	20000919		
	US 2000-247031P	P	20001113		
	US 2000-729995	A	20001206		
	WO 2001-US29161	W	20010919		
	US 2002-135689	A3	20020501		

AB The invention provides protein, cDNA and genomic sequences for a novel **human** protein, which shares sequence homol. to a known kinase protein, and is related to the calcium/calmodulin-dependent protein kinase kinase subfamily. The gene is **expressed** in the eye (**retinoblastomas**) and brain. Forty three novel single nucleotide polymorphism sites (beyond the ORF or in intron regions) were identified. Thus, the present invention specifically provides isolated peptide and nucleic acid mols., methods of identifying orthologs and paralogs of the calcium/calmodulin-dependent protein kinase kinase peptides, methods of identifying modulators of the calcium/calmodulin-dependent protein kinase kinase peptides, and methods of diagnosis and treatment of diseases associated with the calcium/calmodulin-dependent protein kinase kinase.

ST calcium calmodulin dependent protein kinase cDNA sequence homolog **human**

IT Gene, animal
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(encoding calcium/calmodulin-dependent protein **kinase kinase**; **human calcium/calmodulin**
-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Brain
(fetal, gene **expression** in; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Microarray technology
Nucleic acid hybridization
(for detecting calcium/calmodulin-dependent protein kinase kinase gene in a biol. sample; **human** calcium/calmodulin-dependent protein

kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Probes (nucleic acid)
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(for detecting calcium/calmodulin-dependent protein kinase kinase gene in a biol. sample; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Immunoassay
(for detecting calcium/calmodulin-dependent protein kinase kinase in a biol. sample; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Promoter (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(for **expressing** calcium/calmodulin-dependent protein **kinase kinase** homolog; **human calcium/calmodulin**-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT DNA sequences
Drug screening
Human
Molecular **cloning**
Protein sequences
Therapy
cDNA sequences
(**human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Diagnosis
(mol.; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Eye, neoplasm
(retinoblastoma, gene **expression** in; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Genetic polymorphism
(single nucleotide, on calcium/calmodulin-dependent protein **kinase kinase** gene; **human calcium/calmodulin**-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Antibodies and Immunoglobulins
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(to calcium/calmodulin-dependent protein **kinase kinase**; **human calcium/calmodulin**-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT Animal
(transgenic; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT 405947-86-6P
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

IT 405947-87-7P
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(nucleotide sequence; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic

uses thereof)
IT 405947-85-5
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(nucleotide sequence; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)
IT 158886-18-1P
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(sequence homolog; **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)
IT 406154-79-8
RL: PRP (Properties)
(unclaimed protein sequence; protein, gene and cDNA sequences of **human** calcium/calmodulin-dependent protein kinase kinase sequence homolog and diagnostic and therapeutic uses thereof)

```
=> d his

     (FILE 'HOME' ENTERED AT 13:24:46 ON 30 JUL 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 13:25:29 ON 30 JUL 2004
L1       1222343 S KINASE?
L2       2111610 S CALCIUM OR CALMODULIN
L3         21392 S L1(4W)L2
L4            34 S HUMAN (A)L3
L5            28 DUP REM L4 (6 DUPLICATES REMOVED)
L6       6635055 S CLON? OR EXPRESS? OR RECOMBINANT
L7          5864 S L3 AND L6
L8          1850 S HUMAN AND L7
L9          1641 S RETINOBLASTOMAS
L10            1 S L8 AND L9

=> s "375" and glycine
L11          331 "375" AND GLYCINE

=> s l6 and l11
L12           64 L6 AND L11

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13           35 DUP REM L12 (29 DUPLICATES REMOVED)

=> d 1-35 ibib ab

L13   ANSWER 1 OF 35  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-08761  BIOTECHDS
TITLE:            New lipid metabolism proteins and nucleic acids, useful in
                  producing transgenic plants with increased levels of seed
                  storage compound, e.g. lipid, a fatty acid, a starch or a
                  seed storage protein;
                       recombinant protein production via plasmid
                     expression in host cell for use in transgenic
                     plant construction
AUTHOR:           MITTENDORF V; HAERTEL H A; BAUER J; OSWALD O
PATENT ASSIGNEE:  BASF PLANT SCI GMBH
PATENT INFO:      WO 2004013304 12 Feb 2004
APPLICATION INFO: WO 2003-US24364 4 Aug 2003
PRIORITY INFO:    US 2002-400803 2 Aug 2002; US 2002-400803 2 Aug 2002
```

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-157121 [15]

AB DERWENT ABSTRACT:
NOVELTY - An isolated lipid metabolism proteins (LMP) nucleic acid (I) comprising a polynucleotide sequence encoding a polypeptide that functions as a modulator of seed storage compound in a plant, is new.

DETAILED DESCRIPTION - (I) comprises: (a) any of the 41 sequences of 279-2853 bp (ODD SEQ ID NOS: 1-81); (b) a polynucleotide encoding a polypeptide comprising any of the 41 sequences of 92-948 amino acids (EVEN SEQ ID NOS: 2-82); (c) a sequence at least 60 consecutive nucleotides of (a) or (b); (d) a sequence having at least 70%, or 90% sequence identity with (a) or (b); (e) a sequence complementary to (a) or (b); or (f) a sequence that hybridizes under stringent conditions to (a) or (b). INDEPENDENT CLAIMS are also included for: (1) an **expression** vector comprising (I); (2) producing a transgenic plant having a modified level of a seed storage compound; (3) modulating the level of a seed storage compound in a plant; (4) a transgenic plant made by the method of (2), where **expression** of the LMP nucleic acid in the plant results in a modified level of a seed storage compound in the plant as compared to a wild type variety of the plant; (5) a seed produced by the transgenic plant of (4), where the plant **expresses** the LMP polypeptide and where the plant is true breeding for a modified level of the seed storage compound as compared to a wild type variety of the plant; and (6) a seed oil produced by the seed of (5).

BIOTECHNOLOGY - Preferred Vector: The LMP nucleic acid is operatively linked to a heterologous promoter, e.g. a seed-specific promoter, a root-specific promoter or a non-tissue-specific promoter. Preferred Method: Producing a transgenic plant having a modified level of a seed storage compound comprises transforming a plant cell with an **expression** vector comprising (I) and generating from the plant cell the transgenic plant. The level of a seed storage compound is increased in the transgenic plant as compared to the wild type plant. LMP nucleic acid encodes preferably a polypeptide comprising a sequence of 265 amino acids (SEQ ID NO: 28). The modified level of the seed storage compound is due to the overexpression or down-regulation of the LMP nucleic acid. Modulating the level of a seed storage compound in a plant comprises modifying the **expression** of an LMP nucleic acid in the plant, where the LMP nucleic acid comprises a sequence of 301, 458 or 1158 bp (SEQ ID NOS: 1, 5 or 7) or a sequence encoding a polypeptide comprising a sequence of 417, 152 or **375** amino acids (EVEN SEQ ID NOS: 4-8). LMP nucleic acid encodes a polypeptide that contains a DNA-binding domain, protein kinase domain, signal transduction domain, protease domain, lipid metabolism domain and an oxidoreductase domain. Preferred Plant: The plant is a dicotyledonous or a monocotyledonous plant. The plant is an oil producing species, e.g. rapeseed, canola, linseed, soybean, sunflower, maize, oat, rye, barley, wheat, sugarbeet, tagetes, cotton, oil palm, coconut palm, flax, castor or peanut. The seed storage compound is lipid, a fatty acid, a starch or a seed storage protein.

USE - The LMP nucleic acid is useful in producing transgenic plants with increased levels of seed storage compound, e.g. lipid, a fatty acid, a starch or a seed storage protein, as markers for specific regions of the genome and for evolutionary and protein structural studies.

EXAMPLE - Full-length sequences of the Arabidopsis thaliana partial cDNAs (ESTs) were identified and isolated using SMART RACE cDNA amplification kit. The RACE product fragments were extracted from agarose gel and ligated into the TOPO pCR2.1 vector. **Recombinant** vectors were transformed into TOP10 cells. Transformed cells were grown overnight. Single white colonies were selected. Plasmid DNA was extracted and analyses of **clones** and restriction mapping was performed. Gene sequences were used to identify homologous and heterologous genes from cDNA or genomic libraries by using PCR primers. Reverse

transcriptase PCR was performed and restriction sites were added so that RT-PCR products could be **cloned** into restriction sites. The nucleic acid molecule compromises any of the 41 sequences of 279-2853 bp (ODD SEQ ID NOS: 1-81). (115 pages)

L13 ANSWER 2 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-12568 BIOTECHDS
TITLE: New transgenic plant, useful in developing phenotypes with altered or improved characteristics or traits; vector-mediated gene transfer and **expression** in host cell for transgenic plant construction
AUTHOR: ZHANG J; FROMM M E; HEARD J E; RIECHMANN J L; ADAM L J; BROUN P E; PINEDA O; REUBER T L; KEDDIE J S; YU G; JIANG C; SAMAHA R S; PILGRIM M L; CREELMAN R A; DUBELL A N; RATCLIFFE O; KUMIMOTO R; SHERMAN B K
PATENT ASSIGNEE: ZHANG J; FROMM M E; HEARD J E; RIECHMANN J L; ADAM L J; BROUN P E; PINEDA O; REUBER T L; KEDDIE J S; YU G; JIANG C; SAMAHA R S; PILGRIM M L; CREELMAN R A; DUBELL A N; RATCLIFFE O; KUMIMOTO R; SHERMAN B K
PATENT INFO: US 2004045049 4 Mar 2004
APPLICATION INFO: US 2003-412699 10 Apr 2003
PRIORITY INFO: US 2003-412699 10 Apr 2003; US 1999-394519 13 Sep 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-225755 [21]
AB DERWENT ABSTRACT:

NOVELTY - A transgenic plant comprising a **recombinant** polynucleotide having a polynucleotide sequence or its complementary sequence, is new.

DETAILED DESCRIPTION - The transgenic plant comprises a **recombinant** polynucleotide having a polynucleotide sequence or its complementary sequence comprising a sequence: (a) encoding a polypeptide, that initiates transcription, comprising any of the sequences of SEQ ID NOS: 1-1939 or 1944-1973 available in electronic form from the following web site http://seqdata.uspto.gov/sequence.html; Document ID: 20040045049; (b) encoding the polypeptide sequence of (a) with conservative substitutions; (c) a variant, orthologue or paralogue of the sequences of (a), which is at least 80% identical to a sequence of (a); (d) encoding a polypeptide comprising a conserved domain that exhibits at least 80% sequence homology with the conserved domain of the polypeptide of (a) and where the conserved domain of (a) is bounded by amino acid residue coordinates; or (e) that hybridizes to the polynucleotide of (a) under stringent conditions. INDEPENDENT CLAIMS are also included for: (1) using a transgenic plant to grow a progeny plant; (2) an **expression** cassette comprising a constitutive, inducible or tissue-specific promoter and a **recombinant** polynucleotide described above; (3) a host cell comprising the **expression** cassette of (2); (4) producing a modified plant having a modified trait; (5) identifying a factor that is modulated by or interacts with a polypeptide encoded by the polynucleotide sequence; and (6) identifying at least one downstream polynucleotide sequence that is subject to a regulatory effect of any of the polypeptides encoded by the polynucleotide described above.

BIOTECHNOLOGY - Preferred Transgenic Plant: The stringent conditions are 6xSSC and 65 degrees Centigrade. The transgenic plant possesses an altered trait, exhibits an altered phenotype and **expresses** an altered level of one or more genes associated with a plant trait as compared to a non-transformed plant, where the non-transformed plant does not overexpress the **recombinant** polynucleotide. The polynucleotide sequence is derived from a monocotyledonous or dicotyledonous plant. The transgenic plant is dicot or monocot. The plant is a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, clover, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber,

eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, pumpkin, spinach, squash, sweet corn, tobacco, tomato, watermelon, mint and other labiates, rosaceous fruits and vegetable brassicas. The transgenic plant further comprises a constitutive, inducible or tissue-specific promoter operably linked to the polynucleotide sequence or its complement. The encoded polypeptide is **expressed** and regulates transcription of at least one gene. The encoded polypeptide comprises SEQ ID NO: 468. The altered trait is: (a) an enhanced tolerance to abiotic stress, i.e. increased: (i) tolerance to chilling and the nucleotide sequence is SEQ ID NOS: 183, 437 or 805 or their variants; (ii) germination in cold conditions and the nucleotide sequence is SEQ ID NOS: 137, 183, 437, 805 or 841 or their variants; (iii) freezing tolerance and the nucleotide sequence is SEQ ID NO: 615 or its variant; (iv) tolerance to heat and the nucleotide sequence is SEQ ID NOS: 291, 467, 573, 635 or 819 or their variants; (v) tolerance to drought and the nucleotide sequence is SEQ ID NOS: 53, 615 or 941 or their variants; (vi) tolerance to osmotic stress and the nucleotide sequence is SEQ ID NOS: 97, 223, 309, 721, 731 or 941 or their variants; (vii) tolerance to salt and the nucleotide sequence is SEQ ID NOS: 27, 105, 141, 549, 579 or 591 or their variants; (viii) tolerance to phosphate limitation and the nucleotide sequence is SEQ ID NOS: 345 or its variants; or (ix) tolerance to potassium limitation and the nucleotide sequence is SEQ ID NOS: 269, 359 or 613 or their variants or the abiotic stress is decreased sensitivity to nitrogen limitation and the nucleotide sequence is SEQ ID NOS: 139, 141, 467, 805, 939 or 959 or their variants; (b) altered hormone sensitivity, i.e.: (i) reduced sensitivity to abscisic acid and the nucleotide sequence is SEQ ID NOS: 721 or 941 or their variants; or (ii) an altered response to ethylene and the nucleotide sequence is SEQ ID NOS: 507, 713 or 741 or their variants; (c) disease resistance; (d) altered susceptibility to Botrytis and the nucleotide sequence is SEQ ID NOS: 37, 117, 171, 191, 575, 587 or 793 or their variants; (e) altered susceptibility to Fusarium and the nucleotide sequence is SEQ ID NOS: 97, 345 or 595 or their variants; (f) altered susceptibility to Erysiphe and the nucleotide sequence is SEQ ID NOS: 21, 37, 159, 241, 261, 345, 389, 401, 575, 581, 587, 695 or 799 or their variants; (g) altered susceptibility to Pseudomonas syringae and the nucleotide sequence is SEQ ID NOS: 267, 337 or 345 or their variants; (h) altered susceptibility to Sclerotinia and the nucleotide sequence is SEQ ID NOS: 37, 303, 539 or 553 or their variants; (i) altered sugar sensing and the nucleotide sequence is selected from the group consisting of SEQ ID NOS: 33, 41, 49, 117, 137, 163, 179, 193, 217, 343, 369, 463, 529, 531, 579, 605, 615, 659, 719, 831, 861, 911 or 939 or their variants, where altered sugar sensing: (i) is increased tolerance to sugars and the nucleotide sequence is SEQ ID NOS: 137, 529, 531, 579, 605, 911 or 939 or their variants; or (ii) confers improved seed germination and seedling vigor; (j) early flowering and the nucleotide sequence is SEQ ID NOS: 57, 59, 61, 69, 87, 89, 91, 119, 143, 181, 249, 301, 387, 391,405, 539, 575, 685, 787, 819, 867, 921, 937 or ODD SEQ ID NOS: 941-951, 957 or EVEN SEQ ID NOS: 1952-1966 or their variants; (k) late flowering and the nucleotide sequence is SEQ ID NOS: 11, 69, 101, 109, 127, 157, 173, 237, 275, 307, 361, **375**, 389, 463, 487, 489, 497, 503, 567, 585, 603, 615, 639, 657, 699, 723, 745, 859, 887, 893, 901, 911, 1944, 1946, 1948, 1950, 1970 or 1972 or their variants; (l) an extended period of flowering and the nucleotide sequence is SEQ ID NOS: 949 or its variant; (m) altered flower structure and the nucleotide sequence is SEQ ID NOS: 55, 95, 295, 357, 381, 399, 419, 483, 449, 525, 581, 647, 717, 725, 741, 799, 827, 849, 883, 891, 913, 925, 929 or 949 or their variants; (n) an inflorescence architectural change and the nucleotide sequence is SEQ ID NOS: 59, 245, 381, 575, 805, 883, 901 or 913 or their variants; (o) a change in stem bifurcations and the nucleotide sequence is SEQ ID NO: 249 or its variant; (p) a lack of a shoot meristem and the nucleotide sequence is SEQ ID NO: 899 or its variant; (q) reduced meristem cell differentiation and the nucleotide sequence is SEQ ID NO: 919 or its variant; (r) altered phyllotaxy and the nucleotide sequence is SEQ ID NO:

419 or its variant; (s) an altered branching pattern and the nucleotide sequence is SEQ ID NOS: 283 or 913 or their variants; (t) reduced apical dominance and the nucleotide sequence is SEQ ID NOS: 357, 483, 793, 807 or 929 or their variants; (u) reduced trichome density or lack of trichomes and the nucleotide sequence is SEQ ID NOS: 31, 123, 139, 141, 457, 467, 855, 939 or 959 or their variants; (v) ectopic trichome development or altered trichome development and the nucleotide sequence is SEQ ID NO: 169 or its variant; (w) an increase in trichome number and the nucleotide sequence is SEQ ID NO: 415 or its variant; (x) altered stem morphology and the nucleotide sequence is SEQ ID NOS: 283 or 497 or their variants; (y) increased root growth and the nucleotide sequence is SEQ ID NOS: 13 or 905 or their variants; (z) increased root hairs and the nucleotide sequence is SEQ ID NOS: 139,141, 467, 939 or 959 or their variants; (aa) altered seed development and the nucleotide sequence is SEQ ID NO: 649 or its variant; (bb) altered cell proliferation or cell differentiation and the nucleotide sequence is SEQ ID NO: 919 or its variant; (cc) slow growth and the nucleotide sequence is SEQ ID NOS: 285, 491, 713, 859 or 901 or their variants; (dd) premature senescence and the nucleotide sequence is SEQ ID NOS: 417 or 737 or their variants; (ee) delayed senescence and the nucleotide sequence is SEQ ID NOS: 173, **375**, 585 or 697 or their variants; (ff) lethality when overexpressed and the nucleotide sequence is SEQ ID NOS: 239, 379, 583, 727 or 817 or their variants; (gg) increased necrosis and the nucleotide sequence is SEQ ID NO: 29 or its variant; (hh) an increase in seedling or plant size and the nucleotide sequence is SEQ ID NO: 53, 723, 829, 887 or 893 or their variants; (ii) decreased plant size and the nucleotide sequence is SEQ ID NOS: 1, 5, 25, 29, 35, 89,103,185, 203, 245, 285, 417,447, 449, 489, 507, 557, 573, 581, 591, 627, 647, 655, 657, 673, 677, 701, 717, 725, 753, 775, 799, 801, 807, 809, 825, 827, 831, 837, 841, 843, 849, 855, 857, 869, 901, 921 or 925 or their variants; (jj) a change in leaf morphology, i.e. : (i) dark green leaves and the nucleotide sequence is SEQ ID NOS: 245, 285, 615, 627, 647, 737, 801, 843, 851, 857 or 913 or their variants; (ii) altered leaf shape and the nucleotide sequence is SEQ ID NOS: 39, 137, 271, 291, 407, 449, 487, 603, 605, 619, 627, 647, 687, 717, 723, 725, 803, 911 or 929 or their variants; (iii) increased altered leaf development and the nucleotide sequence is SEQ ID NO: 929 or its variant; (iv) increased leaf size and mass and the nucleotide sequence is SEQ ID NOS: 283, 805 or 893 or their variants; (v) glossy leaves and the nucleotide sequence is SEQ ID NOS: 643 or 801 or their variants; or (vi) leaf cell expansion and the nucleotide sequence is SEQ ID NO: 331 or its variant, (kk) change in seed morphology, i.e.: (i) altered seed coloration and the nucleotide sequence is SEQ ID NOS: 67, 435 or 443 or their variants; (ii) increased seed size and the nucleotide sequence is SEQ ID NOS: 115, 385 or 793 or their variants; (iii) decreased seed size and the nucleotide sequence is SEQ ID NO: 749 or its variant; or (iv) altered seed shape and the nucleotide sequence is SEQ ID NOS: 713 or 749 or their variants, (ll) change in leaf biochemistry, i.e.: (i) increased leaf wax and the nucleotide sequence is SEQ ID NOS: 643 or 801 or their variants; (ii) an alteration in leaf prenyl lipid content and the nucleotide sequence is SEQ ID NOS: 127, 203, 653, 739, 845 or 853 or their variants; (iii) increased leaf insoluble sugars and the nucleotide sequence is SEQ ID NOS: 121, 159, 165, 199, 215, 271, 277, 337, 393, 521, 581 or 825 or their variants; (iv) decreased leaf insoluble sugars and the nucleotide sequence is SEQ ID NOS: 215, 271 or 671 or their variants; (v) increased leaf anthocyanins and the nucleotide sequence is SEQ ID NO: 435 or its variant; (vi) an alteration of leaf fatty acid content and the nucleotide sequence is SEQ ID NOS: 127, 151, 473, 799 or 861 or their variants; or (vii) an alteration of leaf glucosinolate content and the nucleotide sequence is SEQ ID NOS: 91,465, 721, 763 or 841 or their variants, (mm) change in seed biochemistry, i.e.: (i) an increase in seed oil content and the nucleotide sequence is SEQ ID NOS: 71, 147, 151, 209, 287, 291, 359, 387, 393, 483, 565, 633, 759 or 763 or their variants; (ii) decrease in seed oil content and the nucleotide sequence is SEQ ID NOS: 87, 101, 111, 135,

163, 435, 443, 473, 483, 521, 613, 843 or 941 or their variants; (iii) increase in seed fatty acid content and the nucleotide sequence is SEQ ID NOS: 523,571, 581, 631, 637, 877 or 881 or their variants;(iv) decrease in seed fatty acid content and the nucleotide sequence is SEQ ID NOS: 519, 541, 631, 653 or 881 or their variants; (v) increase in seed protein content and the nucleotide sequence is SEQ ID NO: 111, 135,141,163,407, 409,435, 443, 473, 483, 575, 613, 695, 843, 891 or 941 or their variants; (vi) decrease in seed protein content and the nucleotide sequence is SEQ ID NOS: 147,151, 267, 287,291,483 or 927 or their variants; (vii) alteration in seed prenyl lipid content and the nucleotide sequence is SEQ ID NOS: 127, 473, 497, 589 or 699 or their variants; (viii) increase in seed sterols and the nucleotide sequence is SEQ ID NO: 23 or its variant; or (ix) upregulation of genes involved in secondary metabolism and the nucleotide sequence is SEQ ID NO: 147 or its variant, (nn) increase in root anthocyanins and the nucleotide sequence is SEQ ID NO: 435 or its variant; (oo) increase in plant anthocyanins and the nucleotide sequence is SEQ ID NOS: 435 or 905 or their variants; or (pp) alteration in light response or shade avoidance and the nucleotide sequence is SEQ ID NOS: 235, 713 or 841 or their variants. Preffered Method: Using a transgenic plant to grow a progeny plant comprises crossing the transgenic plant with itself or another plant, selecting seed that develops as a result of the crossing and growing the progeny plant from the seed. The progeny plant **expresses** mRNA that encodes a DNA-binding protein that binds to a DNA regulatory sequence and induces **expression** of a plant trait gene, the mRNA is **expressed** at a level greater than a non-transformed plant and the progeny plant is characterized by a change in a plant trait compared to the non-transformed plant, where the non-transformed plant does not comprise the **recombinant** polynucleotide. Producing a modified plant having a modified trait comprises selecting a polynucleotide that encodes a polypeptide, described above, inserting the polynucleotide into an **expression** cassette of (2), introducing the **expression** cassette into a plant or a cell of a plant to overexpress the polypeptide, thus producing the modified plant and selecting the modified plant having the modified trait. Identifying a factor that is modulated by or interacts with a polypeptide encoded by the polynucleotide sequence comprises **expressing** a polypeptide encoded by the polynucleotide, described above, in a plant and identifying at least one factor that is modulated by or interacts with the polypeptide. Identifying at least one downstream polynucleotide sequence that is subject to a regulatory effect of any of the polypeptides encoded by the polynucleotide described above comprises **expressing** any of the polypeptides in a plant cell and identifying RNA or protein produced as a result of the **expression** . The identifying is by Northern analysis, RT-PCR, microarray gene **expression** assays, reporter gene **expression** systems subtractive hybridization, differential display, representational differential analysis or by two-dimensional gel electrophoresis of one or more protein products.

USE - The transgenic plant is useful in further developing plants with altered traits.

EXAMPLE - pMEN020 was modified to replace the NptII coding region with the BAR gene of Streptomyces hygroscopicus that conferred resistance to phosphinothricin. The KpnI and BglIII sites of the BAR gene were removed by site-directed mutagenesis with silent codon changes. The **cloning** vector was introduced into a variety of cereal plants, e.g. by direct DNA transfer, Agrobacterium tumefaciens-mediated transformation or by microprojectile. Embryonic cells were derived from immature scutellar tissue by using microprojectile bombardment with the A188XB73 genotype. Tissues were then selected on phosphinothricin to identify the transgenic embryonic cells. Transgenic plants were then regenerated. (213 pages)

L13 ANSWER 3 OF 35 MEDLINE on STN

ACCESSION NUMBER: 2004077803 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14967022
TITLE: Rhodobacter capsulatus photoactive yellow protein: genetic context, spectral and kinetics characterization, and mutagenesis.
AUTHOR: Kyndt J A; Hurley J K; Devreese B; Meyer T E; Cusanovich M A; Tollin G; Van Beeumen J J
CORPORATE SOURCE: Laboratory of Protein Biochemistry and Protein Engineering, University of Gent, Ledeganckstraat 35, 9000 Gent, Belgium.. jkyndt@email.arizona.edu
CONTRACT NUMBER: GM 66146 (NIGMS)
SOURCE: Biochemistry, (2004 Feb 24) 43 (7) 1809-20. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200406
ENTRY DATE: Entered STN: 20040218
Last Updated on STN: 20040624
Entered Medline: 20040622

AB A gene for photoactive yellow protein (PYP) was previously **cloned** from Rhodobacter capsulatus (Rc), and we have now found it to be associated with genes for gas vesicle formation in the recently completed genome sequence. However, the PYP had not been characterized as a protein. We have now produced the **recombinant** RcPYP in Escherichia coli as a glutathione-S-transferase (GST) fusion protein, along with the biosynthetic enzymes, resulting in the formation of holo-RcPYP following cleavage of the GST tag. The absorption spectrum (with characteristic peaks at 435 and **375** nm) and the photocycle kinetics, initiated by a laser flash at 445 nm, are generally similar to those of Rhodobacter sphaeroides (RsPYP) but are significantly different from those of the prototypic PYP from Halorhodospira halophila (HhPYP), which has a single peak at 446 nm and has slower recovery. RcPYP also is photoactive when excited with near-ultraviolet laser light, but the end point is then above the preflash baseline. This suggests that some of the PYP chromophore is present in the cis-protonated conformation in the resting state. The excess 435 nm form in RcPYP, built up from repetitive 365 nm laser flashes, returns to the preflash baseline with an estimated half-life of 2 h, which is markedly slower than that for the same reaction in RsPYP. Met100 has been reported to facilitate cis-trans isomerization in HhPYP, yet both Rc and RsPYPs have Lys and Gly substitutions at positions 99 and 100 (using HhPYP numbering throughout) and have 100-fold faster recovery kinetics than does HhPYP. However, the G100M and K99Q mutations of RcPYP have virtually no effect on kinetics. Apparently, the RcPYP M100 is in a different conformation, as was recently found for the PYP domain of Rhodocista centenaria Ppr. The cumulative results show that the two Rhodobacter PYPs are clearly distinct from the other species of PYP that have been characterized. These properties also suggest a different functional role, that we postulate to be in regulation of gas vesicle genes, which are known to be light-regulated in other species.

L13 ANSWER 4 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-04123 BIOTECHDS
TITLE: Killing gram-positive bacteria in a human patient, useful for treating bacterial infections, comprises contacting the bacteria with a bactericidal-effective amount of mutant human Group II PLA2;
recombinant protein production for use in disease therapy and gene therapy
AUTHOR: WEISS J; ELSBACH P; LIANG N
PATENT ASSIGNEE: UNIV NEW YORK STATE
PATENT INFO: US 2003161822 28 Aug 2003
APPLICATION INFO: US 2002-255576 25 Sep 2002

PRIORITY INFO: US 2002-255576 25 Sep 2002; US 1999-172467 17 Dec 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-897909 [82]
AB DERWENT ABSTRACT:
NOVELTY - Killing gram-positive bacteria in a human patient comprises contacting the bacteria with a bactericidal-effective amount of mutant human Group II PLA2.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) killing Staphylococcus aureus bacteria by contacting the bacteria by a bactericidal-effective amount of mutant Group II PLA2; (2) treating a Staphylococcus aureus infection in a human by administering to the human a bactericidal-effective amount of mutant human Group II PLA2; (3) a pharmaceutical formulation comprising a mutant human Group II PLA2, and a carrier or diluent, where the formulation has a bactericidal activity against Gram-positive bacteria; (4) a purified isolated nucleic acid comprising a sequence of **375** bp, fully defined in the specification; (5) a purified isolated protein comprising a sequence of 124 amino acids fully defined in the specification; and (6) treating a human patient suffering from an infection caused by gram-positive bacteria by administering to a human, a mutant human Group II PLA2, and an antibiotic.

BIOTECHNOLOGY - Preferred Method: In killing Gram-positive bacteria in a human patient, the mutant human Group II PLA2 is genetically altered to produce a **recombinant** protein with amino acid substitutions lysine for **glycine** at residue 72 and lysine for threonine at residue 103. The bacteria is selected are from Micrococcus, Staphylococcus, Streptococcus, Peptococcus, Peptostreptococcus, Enterococcus, Methonobacterium, Bacillus, Clostridium, Lactobacillus, Listeria, Erysipelothrix, Corynebacterium, Propionibacterium, Eubacterium, Actinomyces, Arachnia, Bifidobacterium, Bacterionema, Rothia, Mycobacterium, Nocardia, Streptomyces, and Micropolyspora. Preferred Formulation: The pharmaceutical formulation further comprises an antibiotic. The antibiotic is selected from ampicillin, amoxicillin, oxacillin, cephalosphorins, streptomycin, neomycin, kanamycin, gentamycin, tetracyclines, chloramphenicol, and vancomycin.

ACTIVITY - Antibacterial. S. aureus RN450 (106/ml) was incubated with 1-500 ng/ml of each Group II PLA2 at 37 degreesC for 60 minutes in RMPI-1640 medium containing at 10 mM HEPES, pH 7.4 and 1% (w/v) albumin. Bacterial viability was determined. Results showed that the genetically modified human Group IIA PLA2 has increased bactericidal activity against S. aureus.

MECHANISM OF ACTION - Gene therapy.

USE - The methods and mutant human Group II PLA2 are useful for treating a human suffering from an infection caused by a gram-positive bacteria, e.g. Staphylococcus aureus (claimed).

ADMINISTRATION - Dosage is 1-100 microgram per kg body weight (claimed). Administration is topical, oral, enteral, intravenous, intramuscular, subcutaneous, transdermal, transmucosal, rectal or buccal.

EXAMPLE - No relevant example given. (25 pages)

L13 ANSWER 5 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-18138 BIOTECHDS
TITLE: Novel nucleic acid molecule encoding homologs of Old Yellow Enzyme, particularly those with 12-oxo-phytodienoate reductase activity, useful for transforming plants, increasing stress resistance, and modifying growth; vector-mediated Old-Yellow-Enzyme gene transfer and **expression** in plant host cell for transgenic plant construction and improved stress tolerance
AUTHOR: CRANE V C; DUVICK J; SHARMA Y K; CRASTA O R; FOLKERTS O
PATENT ASSIGNEE: PIONEER HI-BRED INT INC; CURAGEN CORP
PATENT INFO: US 6515202 4 Feb 2003
APPLICATION INFO: US 2000-573906 18 May 2000

PRIORITY INFO: US 2000-573906 18 May 2000; US 1999-134808 19 May 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-465590 [44]
AB DERWENT ABSTRACT:
NOVELTY - An isolated polynucleotide (I) encoding Zea mays Old Yellow Enzyme (ZmOYE) which has a **375** amino acid sequence (S1), and comprising a 1465 base pair sequence (S2), both given in the specification, or an isolated polynucleotide comprising at least 95 % sequence identity to (S2), and encoding a polypeptide comprising 12-oxo-phytodienoate reductase activity or its complement, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an **expression** cassette (II) comprising (I) operably linked to a promoter that drives **expression** in a plant cell; (2) a transformed plant (III) comprising in its genome at least one stably incorporated DNA construct comprising (I) operably linked to a promoter that drives **expression** in a plant cell; (3) transformed seed of (III) comprising the DNA construct; and (4) a transformed plant cell comprising in its genome at least one stably incorporated DNA construct comprising (I) operably linked to a promoter that drives **expression** in a plant cell.

WIDER DISCLOSURE - (1) compositions comprising promoters from genes encoding OYE homologs, for regulating the **expression** of a nucleotide sequence in a plant in response to a stimuli; (2) compositions including antisense sequences, variants and fragments of 12-oxo-phytodienoate reductase genes; (3) compositions comprising promoters of 12-oxo-phytodienoate reductase genes, their synthetic variants and fragments; and (4) proteins encoded by (I).

BIOTECHNOLOGY - Preferred Promoter: The promoter is a constitutive promoter, pathogen-inducible promoter, insect-inducible, wound-inducible, stress-inducible, chemically regulatable, tissue-preferred or developmentally regulated promoter. The promoter is operably linked to the nucleotide sequence for the production of antisense transcripts. Preferred Plant: (III) is a monocotyledon such as maize, wheat, rice, sorghum, barley, millet or rye, or a dicotyledon such as soybean, Brassica sp. alfalfa, safflower, sunflower, cotton or potato.

USE - (I) is useful for transforming plants for constitutive and stress-induced **expression** of sense and antisense sequences for homologs of OYE. (I) finds use in modifying oxylipin metabolism in plants, regulating gene **expression** in plants and in the production of oxylipins in plants. The methods also find use in producing specific oxylipins in plants such as the perfume, methyl jasmonate and jasmonic acid. (I) is further useful for increasing the resistance of a plant to biotic and abiotic stress and for altering plant growth and development processes. The methods find use in improving agronomic traits of agricultural plants. (I) is useful for increasing the resistance or tolerance of plants to stresses caused by organisms, including fungi, bacteria, viruses, nematodes and insects. The oxylipin pathway produces molecules such as anti-pathogenic, volatiles cis-3-hexenol, and trans-2-hexenal and the jasmonates which are involved in defense mechanisms, osmotic regulation, fruit ripening, production of viable pollen, root growth, tendril coiling and senescence. Thus, modulation of the oxylipin pathway modulates these physiological processes. (I) is useful for **expressing** 12-oxo-phytodienoate reductase which is useful for controlling the flow metabolites from the 18-carbon group to the 12-carbon group of the cyclic alpha-linolenic acid derivatives. The **expression** of this enzyme primes or up-regulates the octadecanoid pathway. (I) may also be used in sense orientation to suppress the **expression** of endogenous OYE homolog genes in plants, to isolate corresponding sequences from other organisms, particularly other plants.

EXAMPLE - To identify plant genes **expressed** in response to the initial oxidative burst that was associated with interaction between a resistant host plant and a pathogen, a maize suspension cell model system (Hi-II) was employed. This cell suspension system was selected

because, unlike intact plant tissues, it allowed for the rapid treatment of a uniform population of cells with a equal dosage of a reagent. To mimic the oxidative burst associated with resistant plant-pathogen interactions, the cell suspension was treated with glucose and glucose oxidase for one hour to generate reactive oxygen species. Total RNA was isolated from the cell suspensions and analyzed using CuraGen Corporation's proprietary gene **expression** profiling process (GeneCalling (RTM)) as described in US5871697. A number of distinct transcripts increased in abundance following the oxidative burst and cDNAs corresponding to portion of these transcripts were **cloned** and sequenced. Four of the transcripts that increased in relative abundance to the greatest extent corresponded to sequences encoding Old Yellow Enzyme (OYE) homologous in the Pioneer maize **expressed** sequence tag (EST) database. The increase in abundance of OYE transcripts in maize cell suspensions following an oxidative burst was confirmed by Northern blotting experiments. Two full-length maize ESTs corresponding to the inducible OYE gene homologs were sequenced. The Zea mays OYE1 encoded a **375** amino acid protein, given in the specification. (32 pages)

L13 ANSWER 6 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-01117 BIOTECHDS
TITLE: New fructosyl amino acid oxidase useful in disease diagnosis, oxidizes imino-diacetic acid in presence of oxygen and catalyzes production of glyoxylic acid, alpha-ketoaldehyde, alpha-amino acid and hydrogen peroxide; **recombinant** amino-acid-oxidase production by **expression** in Escherichia coli for use as biocatalyst for glyoxylic acid, alpha-ketoaldehyde, etc., production
PATENT ASSIGNEE: KIKKOMAN CORP
PATENT INFO: JP 2003079386 18 Mar 2003
APPLICATION INFO: JP 2002-181642 21 Jun 2002
PRIORITY INFO: JP 2001-199262 29 Jun 2001; JP 2001-199262 29 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2003-816984 [77]
AB DERWENT ABSTRACT:
NOVELTY - A fructosyl amino acid oxidase (I), that oxidizes imino-diacetic acid or its derivative in the presence of oxygen, catalyzes the reaction involving the production of glyoxylic acid, alpha-ketoaldehyde, alpha-amino acid and hydrogen peroxide and that does not act on epsilon-fructosyl lysine, with an optimum pH of 7.0-8.5, is new.

DETAILED DESCRIPTION - A new fructosyl amino acid oxidase (I), oxidizes imino-diacetic acid or its derivative in the presence of oxygen, catalyzes the reaction involving the production of glyoxylic acid, alpha-ketoaldehyde, alpha-amino acid and hydrogen peroxide, does not act on epsilon-fructosyl lysine, and has an optimum pH of 7.0-8.5. The Km value of (I) against fructosyl **glycine** is 0.31 mM and the optimum temperature range is 25-40 degrees C. At least 80% of the activity of (I) is maintained after heat processing for 10 minutes at 35 degrees C. (I) has a stable pH range of 4.0-8.5. The molecular weight of (I) is 85000 as determined by gel filtration process and 42000 by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). (I) comprises a sequence (S1) of **375** amino acids fully defined in the specification, or a sequence comprising S1 with one or more amino acid deletions, substitutions or additions. INDEPENDENT CLAIMS are also included for: (1) a fructosyl amino acid oxidase gene (II); (2) an **expression** vector (III) comprising (II); (3) a host cell (IV) transfected by (III); and (4) producing (I).

BIOTECHNOLOGY - Preparation: (I) is produced by cultivating (IV) in a culture medium, and recovering (I) from the culture (claimed).

USE - (I) is useful in disease diagnosis (claimed).

EXAMPLE - Total DNA was obtained from Agrobacterium tumefaciens C58, and used as template for PCR amplification using primers AgaP1 and AgaP2. The obtained DNA was digested by EcoRV and subcloned into pUC19 vector. The transformed vector was introduced into Escherichia coli JM109 cells. The transformed cells were cultured and the plasmid was extracted. A limited enzyme treatment of the plasmid was carried out to isolate a DNA fragment of required size. An AgaE homolog A gene was introduced in the same orientation as that of the luc promoter in pUC19 to produced vector pUC-AgaEH. Sequence analysis of the obtained plasmid was carried out and the length of the sequence was found to be 1125 nucleotides fully defined in the specification. pUC-AgaEH was introduced into E. coli JM109, and the transformed cells were cultured in Luria-Bertani (LB)-amp culture medium at 30 degrees C for 24 hours. The cells were centrifuged after culture, and a crude enzyme liquid was obtained from the supernatant. The protein solution was subjected to purification process.(11 pages)

L13 ANSWER 7 OF 35 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:97550 HCAPLUS
DOCUMENT NUMBER: 138:164674
TITLE: Molecular markers for hepatocellular carcinoma and their use in diagnosis and therapy
INVENTOR(S): Debuschewitz, Sabine; Jobst, Juergen; Kaiser, Stephan
PATENT ASSIGNEE(S): Germany
SOURCE: PCT Int. Appl., 98 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003010336	A2	20030206	WO 2002-EP8305	20020725
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
DE 10136273	A1	20030213	DE 2001-10136273	20010725
WO 2004011945	A2	20040205	WO 2003-EP8243	20030725
WO 2004011945	A3	20040603		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: DE 2001-10136273 A 20010725
WO 2002-EP8305 A 20020725

AB The invention relates to mol. markers occurring for hepatocellular carcinoma. The invention more particularly comprises gene sequences or peptides coded thereby which can be regulated upwards or downwards for hepatic cell carcinoma (HCC) in relation to healthy, normal liver cells in

the **expression** thereof. The invention also relates to the use of said sequences in the diagnosis and/or therapy of HCC and for screening purposes in order to identify novel active ingredients for HCC. The invention also relates to an HCC specific cluster as a unique diagnostic agent for HCC.

L13 ANSWER 8 OF 35 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2003:768801 SCISEARCH
THE GENUINE ARTICLE: 717FU
TITLE: beta-Alanine N-methyltransferase of Limonium latifolium. cDNA **cloning** and functional **expression** of a novel N-methyltransferase implicated in the synthesis of the osmoprotectant beta-alanine betaine
AUTHOR: Raman S B; Rathinasabapathi B (Reprint)
CORPORATE SOURCE: Univ Florida, Dept Hort Sci, POB 110 690, Gainesville, FL 32611 USA (Reprint); Univ Florida, Dept Hort Sci, Gainesville, FL 32611 USA
COUNTRY OF AUTHOR: USA
SOURCE: PLANT PHYSIOLOGY, (JUL 2003) Vol. 132, No. 3, pp. 1642-1651.
Publisher: AMER SOC PLANT BIOLOGISTS, 15501 MONONA DRIVE, ROCKVILLE, MD 20855 USA.
ISSN: 0032-0889.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB beta-alanine (Ala) betaine, an osmoprotectant suitable under saline and hypoxic environments, is found in most members of the halophytic plant family Plumbaginaceae. In Limonium latifolium (Plumbaginaceae), it is synthesized via methylation of beta-Ala by the action of a trifunctional S-adenosyl L-methionine (Ado-Met): beta-Ala N-methyltransferase (NMTase). Peptide sequences from purified beta-Ala NMTase were used to design primers for reverse transcriptase-PCR, and several cDNA **clones** were isolated. The 5' end of the cDNA was **cloned** using a 5'-rapid amplification of cDNA ends protocol. A 500-bp cDNA was used as a probe to screen a lambda-gt10 L. latifolium leaf cDNA library. Partial cDNA **clones** represented two groups, NMTase A and NMTase B, differing only in their 3'-untranslated regions. The full-length NMTase A cDNA was 1,414 bp and included a 1128-bp open reading frame and a 119-bp 5'-untranslated region. The deduced amino acid sequence of **375** residues had motifs known to be involved in the binding of Ado-Met. The NMTase mRNA was **expressed** in L. latifolium leaves but was absent in Limonium sinuatum, a member of the genus that lacks the synthetic pathway for beta-Ala betaine. NMTase mRNA **expression** was high in young and mature leaves and was enhanced by light. NMTase cDNA was **expressed** in yeast (Saccharomyces cerevisiae) under the control of a galactose-inducible promoter. Protein extracts of galactose-induced **recombinant** yeast had Ado-Met-specific NMTase activities that were highly specific to beta-Ala, N-methyl beta-Ala, and N,N-dimethyl beta-Ala as methyl acceptors. NMTase activities were not detectable in comparable protein extracts of yeast, transformed with vector control. The NMTase protein sequence shared homology with plant caffeic acid O-methyltransferases and related enzymes. Phylogenetic analyses suggested that beta-Ala NMTase represents a novel family of N-methyltransferases that are evolutionarily related to O-methyltransferases.

L13 ANSWER 9 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-08692 BIOTECHDS
TITLE: Novel dimeric protein comprising a first fusion protein which comprises targeting domain, leucine zipper domain and antigen, and a second fusion protein which comprises targeting domain, leucine zipper domain;
vector-mediated **recombinant** protein gene

transfer and **expression** in host cell for use in **recombinant** vaccine preparation and cancer, virus, bacterium and parasitic infection therapy

AUTHOR: VALIANTE N
PATENT ASSIGNEE: CHIRON SPA
PATENT INFO: WO 2002098456 12 Dec 2002
APPLICATION INFO: WO 2002-IB3105 30 May 2002
PRIORITY INFO: GB 2001-13798 6 Jun 2001; GB 2001-13798 6 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-140555 [13]

AB DERWENT ABSTRACT:

NOVELTY - A dimeric protein (I) comprising a first fusion protein (comprising a targeting domain, leucine zipper domain and an antigen) and a second fusion protein (comprising a targeting domain, leucine zipper domain and optionally an antigen), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fusion protein (II) comprising, a targeting domain, leucine zipper domain and antigen; (2) a nucleic acid vector (III) encoding (I); (3) a nucleic acid vector (IV) encoding (II); (4) a composition (V) comprising two nucleic acid vectors (III) and (IV), which encode (I); (5) a host cell (VI) comprising (III) and/or (IV); (6) a pharmaceutical composition (VII) comprising (III), (IV), or (IV); and (7) polyclonal or monoclonal antibodies obtained by administering (III), (IV), (V) or (VII) to a subject in vivo or to cells or tissues in vitro.

WIDER DISCLOSURE - Kits comprising (III) and/or (IV) are also disclosed.

BIOTECHNOLOGY - Preferred Dimeric protein: (I) is targeted to antigen presenting cells via the targeting domains. The targeting domains in the first and the second fusion proteins are the same or different. The same targeting domains comprise cytotoxic T lymphocyte associated antigen-4 (CTLA-4) or its fragment (e.g., the V-domain of CTLA-4). The different targeting domains comprise an antibody. The leucine zipper domains in the first fusion protein and the second fusion protein are the same, where both leucine zipper domains are derived from the transcription factor GCN4. Optionally, the leucine zipper domains in the first and second fusion proteins are different, where one leucine zipper domain is derived from the transcription factor fos and the other is derived from the transcription factor jun. The antigen in the first and second fusions are the same or different. Optionally, the second fusion protein does not comprise an antigen. The antigens are selected from tumor antigens, bacterial antigens, viral antigens or parasitic antigens. Preferably (I) comprises hepatitis C virus (HCV) antigens, where the antigen in the first fusion protein is HCV-E1 and the antigen in the second fusion protein is HCV-E2. Preferred Vector: In (III) or (IV), a secretory sequence, a leader sequence or a retention sequence is fused to the nucleotide sequence encoding targeting proteins. Preferably, the sequence is chosen from tissue-type human plasminogen activator signal/pro sequence, the endoplasmic reticulum retention sequence KDEL, and the glycosylphosphatidylinositol (GPI) anchor signal.

ACTIVITY - Virucide; Antibacterial; Antiparasitic; Cytostatic. No biological data given.

MECHANISM OF ACTION - Vaccine.

USE - (III), (IV), (V) or (VII) is useful in therapy, or in the manufacture of a medicament for stimulating a specific response to one or more antigens chosen from tumor antigens, bacterial antigens, viral antigens or parasitic antigens, preferably hepatitis C virus (HCV) antigens, where the antigen in the first fusion protein is HCV-E1 and the antigen in the second fusion protein is HCV-E2. (III), (IV), (V) or (VII) is useful for stimulating a specific immune response to one or more antigens, in a subject, and also for stimulating a specific immune response to one or more antigens, in cells or tissues, in vitro (claimed). (VII) is useful as vaccine. The vectors and pharmaceutical compositions are useful for producing increased yields of antibodies in

vitro.

ADMINISTRATION - (VII) useful as vaccines are administered by parenteral, e.g., by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously. No specific clinical dosages are given.

ADVANTAGE - The vectors and pharmaceutical compositions allow the production of antibodies to an antigen when the only information known about the antigen is its gene sequence.

EXAMPLE - Construction of DNA vaccination vector Pac-FN-NF (starting point vector) was carried out as follows. Mammalian reporter vector pCMVbeta was digested with NotI to remove the beta-galactosidase reporter gene. The linearized vector was purified, resulting in the vector pCMV-N. To introduce multiple **cloning** sites (MCS), oligonucleotides were generated and purified. Two oligomers of 71 nucleotides length (mcs1 (5'-GGCCGCACGCGTACTAGTGGGCCCGGGCGTACGCTTAAGAATCGATATC GGTACCAGATCTGAATTCGGCC-3') and mcs2 (5' -GGCCGGCCGAATTCAGATCTGGTACCGATATC GATTCTTAAGCGTACGCCCGGG CCCACTAGTACGCGTGC-3') 8 were annealed at equimolar conditions in T4 ligase buffer by heating to 70 degreesC for 5 minutes (min), slowly cooling down to 30 degreesC and a final chill on ice. The resulting double stranded MCS (MCS-FN/NF)) with 5'-GGCC-overhangs was inserted into the NotI site of pCMV-N. The mcs1 oligomer was labeled with (gamma32P)-ATP by polynucleotide kinase and used to identify positive **clones** by colony hybridization. **Clones** were tested for the direction of the MCS by restriction digestions. The resulting vectors were designated pCMV-FN and pCMV-NF. The pUC19 ori was amplified by polymerase chain reaction (PCR) on pCMVbeta, and the PCR product was digested with SalI and HindIII. The asd gene of Salmonella typhimurium was amplified by PCR on pYA3137. The PCR product was digested with HindIII and PstI. Both pCMV-FN and pCMV-NF were digested with PstI and SalI and the eukaryotic **expression** cassettes of 1086 bp length were purified. The digested PCR products ori (675 bp)and asd (1739 bp) were purified on MicroSpin S-40 HR columns and ligated to the 1086 bp fragments. The resulting plasmids were referred to as pAC-FN and pAC-NF (3500 bp). Construction of pAC-AH was carried out as follows. A murine immnuostimulatory sequence (ISS) of 30 bp length was introduced into the HindIII site of pAC-FN. Annealing of the oligonucleotides iss1 and iss2 (5' -AGCTAGCTAGCAACGTCAGGAACGTCATGGAT-3') (5' - AGCTATCCATGACGTTCGACGTTGCTAGCT -3') resulted in a HindIII-compatible 5'-AGCT overhang. To facilitate screening, the HindIII site is eliminated upon insertion of the double stranded oligonucleotide, and a unique NheI site is introduced. The resulting plasmid of 3532 bp length was designated pAC-FN-ISS. in a second step, the V-like domain of mouse cytotoxic T lymphocyte associated antigen-4 (CTLA-4) was amplified by RT-PCR from splenocytes of a female C57BL/6 mouse. The cells were resuspended by passage through a Falcon 70 micrometer mesh size nylon cell strainer and further purified. 5000000 cells were cultured per well of a six-well tissue culture cluster in 3 ml RPMI-1640, 2 mM L-glutamine, 100 units penicillin per ml. 100 microliters streptomycin per ml. 10% FBS and 100 units of **recombinant** human interleukin-2 per ml for 24 hours at 37degreesC and 9% CO2 atmosphere 40 x 106 cells were washed with PB, and RNA was extracted. First strand cDNA was generated. 2 mug of total RNA were annealed to 500 ng of oligo(dT)12-18 primer. First strand synthesis was performed for 50 minutes at 42 degrees C with 200 units Superscript II reverse transcriptase in first strand buffer (0.1 M dithiotheritol (DTT)-10 mM each dNTP). The enzyme was subsequently inactivated and RNA was digested for 20 minutes 37degreesC with 1 micrograms RNAse A. The V-like domain of mouse CTLA-4 was amplified by PCR using one tenth of the generated first strand cDNA as template, generating a fragment of **375** bp length. In a third step, the mouse CTLA-4 domain was fused on the 5'-end to the tissue plasminogen activator (tPA) signal sequence and on the 3'-end via **glycine** linkers to the GCN4 leucine zipper and to a new MCS. This was achieved by overlapping PCR amplifications. The final 697 bp product was **cloned** into the FseI and NotI sites of Pac-FN-ISS, and the

resulting construct was designated the DNA vaccination vector Pac-AH (final vector).(30 pages)

L13 ANSWER 10 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-02217 BIOTECHDS
TITLE: New genetic variants having polymorphisms in the Tachykinin receptor (TACR2) protein, useful for studying the function of TACR2, and for treating disorders associated with abnormal **expression** or function of TACR2 isogene; vector-mediated **recombinant** protein gene transfer and **expression** in host cell for use in drug screening and cancer and asthma gene therapy
AUTHOR: CAPPOLA G; CHEW A; GILSON C R; KOSHY B
PATENT ASSIGNEE: GENAISSANCE PHARM INC
PATENT INFO: WO 2002063046 15 Aug 2002
APPLICATION INFO: WO 2001-US47394 9 Nov 2001
PRIORITY INFO: US 2000-247649 9 Nov 2000; US 2000-247649 9 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-636600 [68]
AB DERWENT ABSTRACT:
NOVELTY - An isolated polypeptide comprising a polymeric variant of a reference sequence for the Tachykinin receptor (TACR2) protein, is new. It is a 398 residue amino acid sequence (S1), given in the specification, for the regions encoded by exons 1, 4 and 5, except that it comprises variant amino acids consisting of **glycine** at position 5, threonine at 23 or 47, alanine at 251 or 363, histidine at **375**, and arginine at 395.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) haplotyping or genotyping the TACR2 gene of an individual; (2) predicting a haplotype pair for the TACR2 gene of an individual; (3) identifying an association between a trait and at least one haplotype or haplotype pair of the TACR2 gene; (4) isolated oligonucleotide for detecting a polymorphism in the TACR2 gene at a polymorphic site (PS) selected from PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PS9, PS10, PS11, PS12, PS13, PS14, PS15, PS16, PS17, PS18, PS19, PS20, PS21, PS22, PS23, PS24, PS25, PS26, PS27 and PS28, where the selected PS and alternative alleles are located in a 17077 base pair sequence (I), given in the specification; (5) a kit for genotyping or haplotyping the TACR2 gene of an individual, comprising a set of oligonucleotides designed to haplotype or genotype each of PS1-PS28, where the selected PS have the location and alternative alleles of (I); (6) an isolated polynucleotide comprising: (a) a first nucleotide sequence consisting of TACR2 isogene selected from isogenes 1-11 and 13-25, where each isogene comprises a region of (I), and is further defined by the corresponding sequence of polymorphisms whose positions and identities are given in the specification; and (b) a second nucleotide sequence which is a complement of (a); (7) an isolated polynucleotide comprising a coding sequence for a TACR2 isogene, which comprises the regions of a 1197 base pair sequence (II), given in the specification defined by exons 1, 4 and 5, except at each of the polymorphic sites having the positions in the above sequence, and polymorphisms given in the specification; (8) **recombinant** nonhuman organisms transformed or transfected with the isolated polynucleotide in (7), where the organism **expresses** a TACR2 protein encoded by the polymorphic variant sequence; (9) an isolated fragment of TACR2 coding sequence, comprising one or more polymorphisms of guanine at nucleotide position 14, 751, 1087 or 1184, cytosine at position 68, adenine at position 139 or 1124, and thymine at position 1128 of sequence (II), or an isolated fragment of TACR2 protein comprising one or more variant amino acids of **glycine** at position 5, threonine at position 23 or 47, alanine at 251 or 363, histidine at **375**, and arginine at 395 in (S1); (10) an isolated monoclonal antibody specific for and immunoreactive with the isolated polypeptide; (11) screening drugs targeting the above polypeptide; (12) a

computer system for storing and analyzing polymorphism data for the TACR2, comprising: (a) a central processing unit; (b) a communication interface; (c) a display device; (d) an input device; and (e) a database containing the polymorphism data which comprises the haplotypes fully described in the specification; and (13) a genome anthology for the TACR2 gene comprising 2 or more TACR2 isogenes selected from isogenes 1-25 as defined in the specification, each comprising a region of (I) and is further defined by the corresponding sequence of polymorphisms whose positions and identities also given in the specification.

BIOTECHNOLOGY - Preferred Method: Haplotyping the TACR2 gene of an individual comprises determining which of the TACR2 haplotypes fully defined in the specification defines one copy of the individual's TACR2 gene, where each of the TACR2 haplotypes comprises a set of polymorphisms whose locations and identities are fully described in the specification. Alternatively, the method comprises determining which of the TACR2 haplotype pairs given in the specification defines both copies of the individual's TACR2 gene, where each of the TACR2 haplotype pairs consists of first and second haplotypes which comprise first and second sets of polymorphisms whose locations and identities are fully described in the specification. Determining which of the TACR2 haplotypes defines one copy of the individual's TACR2 gene comprises identifying the phased sequence of nucleotides present at each of PS1-PS28 on the copy of the individual's TACR2 gene. Furthermore, the method comprises determining, for one copy of the TACR2 gene, the identity of the nucleotide at two or more PS selected from PS1-PS28, having the position and alternative alleles shown in (I). Genotyping the TACR2 gene of an individual comprises determining for the two copies of the TACR2 gene present in the individual the identity of the nucleotide pair at one or more polymorphic sites (PS) selected from PS1-PS28, where the PS have the location and alternative alleles shown in (I). Determining for the 2 copies of the TACR2 comprises: (a) isolating from the individual a nucleic acid mixture comprising both copies of the TACR2 gene or its fragment present in the individual; (b) amplifying from the nucleic acid mixture a target region containing the selected polymorphic site; (c) hybridizing a primer extension oligonucleotide to one allele of the amplified target region; (d) performing a nucleic acid template-dependent, primer extension reaction on the hybridized oligonucleotide in the presence of at least one terminator of the reaction, where the terminator is complementary to the alternative nucleotides present at the selected polymorphic site; and (e) detecting the presence and identity of the terminator in the extended oligonucleotide. The method also comprises determining for the two copies of the TACR2 gene present in the individual the identity of the nucleotide pair at each of PS1-PS28. Predicting a haplotype pair for the TACR2 gene of an individual comprises: (a) identifying a TACR2 genotype for the individual, where the genotype comprises the nucleotide pair at two or more polymorphic sites selected from PS1-PS28, and the selected PS have the position and alternative alleles in (I); (b) comparing the genotype to the haplotype pair data fully defined in the specification; and (c) determining which haplotype pair is consistent with the genotype of the individual and the haplotype pair data. The identified genotype of the individual comprises the nucleotide pair at PS1-PS28, having the location and alternative alleles shown in (I). Identifying an association between a trait and at least one haplotype or haplotype pair of the TACR2 gene comprises comparing the frequency of the haplotype or haplotype pair in a population exhibiting the trait with the frequency of the haplotype or haplotype pair in a reference population, where the haplotype is selected from haplotypes 1-25 fully defined in the specification and each of the haplotypes comprises a set of polymorphisms whose locations and identities are also described in the specification. The haplotype pair is selected from haplotype pairs listed in the specification, where each of the TACR2 haplotype pairs consists of a first and second haplotypes which comprises first and second sets of polymorphisms whose locations and identities are also given in the specification. A higher frequency of the haplotype or haplotype pair in the trait population than in the reference

population indicates that the trait is associated with the haplotype or haplotype pair. The trait is a clinical response to a drug targeting TACR2 or to a drug for treating a condition or disease associated with TACR2 activity. Preferred Oligonucleotide: The allele-specific oligonucleotide specifically hybridizes to an allele of the TACR2 gene at a region containing the polymorphic site. The oligonucleotide is a primer-extension oligonucleotide, and comprises a sequence selected from any of the 55 fully defined sequences, each comprising 10 bp, given in the specification. Preferred **Recombinant** Organism: The **recombinant** nonhuman organism is a transgenic animal.

ACTIVITY - Antiasthmatic; Cytostatic. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - Polymorphic variants of the TACR2 gene are useful in studying the **expression** and biological function of TACR2, and in identifying drugs targeting TACR2 protein for treating disorders associated with abnormal **expression** or function of TACR2, e.g. asthma or breast cancer. Polynucleotides comprising a polymorphic gene variant or fragment may be used for therapeutic purposes, where a patient could benefit from **expression** or increased **expression** of a particular TACR2 protein isoform, or an **expression** vector encoding the isoform may be administered to the patient. Haplotype information is useful in improving the efficiency and output of several steps in drug discovery and development process, including target validation, identifying lead compounds, and early phase clinical trials. Information on polymorphisms may be applied in studying biological functions of TACR2 as well as in identifying drugs targeting this protein for the treatment of disorders related to its abnormal **expression** or function.

ADMINISTRATION - Administration can be oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, intradermal, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal. No dosage is given.

EXAMPLE - Target regions of the TACR2 gene were amplified using polymerase chain reaction (PCR) primers containing genomic DNA isolated from immortalized cell lines for each member of the Index Repository. Amplification profile consisted of 1 cycle at 97 degrees C for 2 minutes; 10 cycles at 97 degrees C for 15 s, 70 degrees C at 45 s, and at 72 degrees C for 45 s; and 35 cycles each at 97 degrees C for 15 s, at 64 degrees C for 45 s, and 72 degrees C for 45 s. PCR products were purified using a Whatman/Polyfiltronics 384 well unifilter (100 micro-l), and purified DNA was eluted in distilled water (50 micro-l). Sequencing reactions were set up using Applied Biosystems Big Dye Terminator chemistry. The purified PCR products were sequenced in both directions using primer sets, and reaction products were purified by isopropanol precipitation, and run on an Applied Biosystems 3700 DNA Analyzer. Sequences were analyzed for the presence of polymorphism using the Polyphred program, and the presence of a polymorphism was confirmed on both strands.(130 pages)

L13 ANSWER 11 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01781 BIOTECHDS
TITLE: **Recombinant** fragment of the surface protein gp195 polypeptide from Plasmodium falciparum, useful to immunize against the human malaria pathogen, is **expressed** from a baculovirus vector in insect cells; baculo virus vector-mediated **recombinant** protein gene transfer and **expression** in Spodoptera frugiperda, Spodoptera exiaua, Spodoptera littoralis, Choristoneura fumiferana or Trichoplusia ni for use in **recombinant** vaccine preparation against human malaria infection
AUTHOR: CHANG S; HUI G S N; BARR P J; GIBSON H
PATENT ASSIGNEE: UNIV HAWAII

PATENT INFO: US 6420523 16 Jul 2002
APPLICATION INFO: US 1994-195705 14 Feb 1994
PRIORITY INFO: US 1994-195705 14 Feb 1994; US 1992-867768 13 Apr 1992
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-626087 [67]
AB DERWENT ABSTRACT:
NOVELTY - A composition comprising a polypeptide **expressed** by an insect cell which contains a baculovirus vector encoding a fragment of the surface protein gp195 (p42) from Plasmodium falciparum, where the polypeptide is more immunogenic in a mammalian host than the same polypeptide **expressed** in yeast, is new.

DETAILED DESCRIPTION - A composition comprising a polypeptide **expressed** by an insect cell which contains a baculovirus vector encoding the polypeptide, where the polypeptide consists of a fragment of the surface protein gp195 from Plasmodium falciparum having residues: (a) 1-373 or 1-394 of the 394 amino acid sequence fully defined in the specification; (b) 1-354 or 1-**375** of the **375** amino acid sequence fully defined in the specification; (c) 1-373 or 1-394 of the 394 amino acid sequence fully defined in the specification; or (d) 1-356 or 1-377 of the 377 amino acid sequence fully defined in the specification, where the polypeptide is more immunogenic in a mammalian host than a polypeptide having the same amino acid sequence **expressed** in yeast.

BIOTECHNOLOGY - Preferred Composition: The insect cells in which the polypeptide is **expressed** are preferably Spodoptera frugiperda, S. exiaua, S. littoralis, Choristoneura fumiferana, or Trichoplusia ni. The composition comprises 0.2 - 5 mg polypeptide. The composition preferably further comprises an adjuvant which is Freund's complete, Freund's incomplete, B30-MDP, LA-15-PH, saponin and/or aluminum hydroxide.

ACTIVITY - Antimalarial.

MECHANISM OF ACTION - Vaccine. Rabbits were immunized with the insect cell-produced, baculovirus encoded gp195 fragment (BVp42), yeast-produced p42 (Yp42) or enriched gp195 mixture (enriched for gp195 and C-terminal containing processing fragments through Mab 5.2 affinity purification) to determine the immunogenicity of the **recombinant** polypeptides and the cross-reactivity of anti-**recombinant** p42 antibodies with native gp195. Doses were 50, then 25, then 12.5 micrograms purified polypeptide in Freund's complete adjuvant given intramuscularly five times at 21 day intervals. Rabbits were bled before immunization and weekly after each immunization. Serum antibodies produced against enriched gp195, BVp42 or Tp42 were assayed by enzyme-linked immunosorbent assay (ELISA) using standard technique. The highest ELISA titers were obtained against parasite gp195, followed by BVp42 then Yp42.

USE - The composition is used as a vaccine to protect against infection by the human malaria pathogen Plasmodium falciparum. The fragment of gp195 is the 42 kDa C-terminal processing fragment known as p42.

EXAMPLE - A mixture of gp195 protein and certain of its processing fragments was obtained from in vitro cultured parasites (Plasmodium falciparum Uganda Palo Alto strain) using monoclonal-antibody affinity chromatography procedures employing Nab 5.2(7); i.e. the mixture is enriched in the epitope for which Mab 5.2 is specific. Importantly, this mixture has been previously shown to be capable of inducing substantially complete protection against a homologous challenge of Plasmodium falciparum in Aotus monkeys. References in the example below to gp195 refer to the Mab 5.2-purified mixture of gp195 enriched with certain of its processing fragments. In summary, saponin-lysed parasites were extracted with 1% NP-40 and the lysate was clarified by ultracentrifugation. The extracts were passed through a Protein G Sepharose column covalently conjugated with gp195-specific monoclonal antibody Mab 5.2 (described in U.S. Pat. Number 4,897,354, incorporated by

reference herein, and deposited with the American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD. 20852, U.S.A. on Jul. 17, 1986 under accession number HB 9148). Mab 5.2 is specific for an antigenic determinant contained in p42, as demonstrated below. After extensive washing to remove non-specifically bound material, specifically-bound proteins were eluted with 0.1 M **glycine** (pH 2.5) and neutralized with 1 M Tris-HCl (pH 8.0). The purity of the isolated gp195 was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining.(13 pages)

L13 ANSWER 12 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002271166 EMBASE
TITLE: Genetic polymorphism of the human cytochrome P450 CYP4B1: Evidence for a non-functional allelic variant.
AUTHOR: Lo-Guidice J.-M.; Allorge D.; Cauffiez C.; Chevalier D.; Lafitte J.-J.; Lhermitte M.; Broly F.
CORPORATE SOURCE: Dr. J.-M. Lo-Guidice, Equipe d'accueil 2679, Faculte de Medecine de Lille, Pole Recherche, Place de Verdun, 59045 Lille, France. jmlo.guidice@univ-lille2.fr
SOURCE: Pharmacogenetics, (2002) 12/5 (367-374).
Refs: 33
ISSN: 0960-314X CODEN: PHMCEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB In the present study, we report the first systematic investigation of polymorphism in the human CYP4B1 gene. Using a strategy based on single-strand conformation polymorphism analysis of PCR products (PCR-SSCP), we analyzed the twelve exons of the gene, as well as their 5'- and 3'- proximal flanking sequences, in DNA samples from 190 French Caucasians. In addition to the wild-type CYP4B1* allele (CYP4B1* 1), four variants, namely CYP4B1* 2, *3, *4 and *5, were characterized. The CYP4B1* 3, *4 and *5 alleles encode missense mutations Arg(173)Trp, Ser(322)Gly and Met(331)Ile, respectively. The fourth variant, CYP4B1* 2, harbors three missense mutations (Met(331)Ile, Arg(340)Cys and Arg(**375**)Cys) and a double nucleotide deletion (AT881-882del) that causes a frameshift and premature stop codon in the second third of the coding sequence of the gene. This latter mutation can be assumed to lead to the synthesis of a severely truncated protein and, therefore, probably contributes to interindividual variability of CYP4B1 **expression** and enzymatic activity. In order to investigate the extent of the CYP4B1* 2 allele in a large population, a rapid genotyping test, based on restriction analysis of PCR products, was developed and applied to 2082 French Caucasians. Forty-two subjects were found homozygous for the AT881-882 deletion, which suggests that about 2% of individuals should be unable to develop metabolic reactions mediated by CYP4B1. Given the relatively high frequency and the functional consequences of the CYP4B1* 2 allele, associations between CYP4B1 polymorphism and certain pathological processes should be considered. .COPYRGT. 2002 Lippincott Williams & Wilkins.

L13 ANSWER 13 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:324761 BIOSIS
DOCUMENT NUMBER: PREV200300324761
TITLE: CRITICAL EPITOPES OF A NR1 VACCINE FOR STROKE AND EPILEPSY.
AUTHOR(S): Symes, C. W.; Jiao, X. Y. [Reprint Author]; Young, D.; Klugmann, M.; Oshinsky, M. L. [Reprint Author]; During, M. J. [Reprint Author]
CORPORATE SOURCE: Neurosurgery, Thomas Jefferson University, Philadelphia, PA, USA
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary

Planner, (2002) Vol. 2002, pp. Abstract No. 600.4.
http://sfn.scholarone.com. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience. Orlando, Florida, USA. November 02-07, 2002. Society for Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; (Meeting Poster)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Jul 2003
Last Updated on STN: 16 Jul 2003

AB The NMDA receptor is implicated in neuropathological states including stroke and epilepsy. We have recently described a genetic vaccine targeting the NR1 subunit of the receptor with prophylactic efficacy in both these conditions (Science 287, 1453;2000). To further elucidate the mechanism of neuroprotection we have attempted to define the critical target epitopes. **Recombinant** proteins to 3 different functional NR1 regions were tested in two rat models of epilepsy, systemic & local intrahippocampal kainate. Antigens corresponding to NR1(21-**375**), the region containing the R1R2 domain, and NR1(654-800), the extracellular loop between TM3 and TM4 that contains the S2 lobe implicated in **glycine** binding, both exerted anti-epileptic effects in terms of latency and seizure progression. Conversely an antigen corresponding to NR1(313-619) showed signs of pro-convulsive activity. TUNEL staining showed that NR1(654-800) protected against hippocampal cell death whereas NR1(313-619) potentiated the neuronal damage compared to naive and control vaccinated rats. NR1(21-**375**) vaccinated rats had typical damage as in the naive rats. All 3 proteins showed similarly efficacy as antigens inducing high titer antibodies with no clear relationship between titer and neuroprotective (or proconvulsant) phenotype with a threshold effect likely. These data therefore further support the concept of targeted autoimmunity to protect the brain against insults with the corollary that antigens need be chosen carefully, and within a given protein both protective and injurious epitopes are likely to co-exist.

L13 ANSWER 14 OF 35 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003458054 EMBASE
TITLE: Phosphorylation of Ser(363), Thr(370), and Ser (**375**) Residues within the Carboxyl Tail Differentially Regulates μ-Opioid Receptor Internalization.
AUTHOR: El Kouhen R.; Burd A.L.; Erickson-Herbrandson L.J.; Chang C.-Y.; Law P.-Y.; Loh H.H.
CORPORATE SOURCE: R. El Kouhen, Dept. of Pharmacology, Univ. of Minnesota Medical School, 6-120 Jackson Hall, 321 Church St., S.E., Minneapolis, MN 55455, United States. elkou001@tc.umn.edu
SOURCE: Journal of Biological Chemistry, (20 Apr 2001) 276/16 (12774-12780).
Refs: 52
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Prolonged activation of opioid receptors leads to their phosphorylation, desensitization, internalization, and down-regulation. To elucidate the relationship between μ-opioid receptor (MOR) phosphorylation and the regulation of receptor activity, a series of receptor mutants was constructed in which the 12 Ser/Thr residues of the COOH-terminal portion of the receptor were substituted to Ala, either individually or in combination. All these mutant constructs were stably **expressed** in human embryonic kidney 293 cells and exhibited similar

expression levels and ligand binding properties. Among those 12 Ser/Thr residues, Ser(363), Thr(370), and Ser (**375**) have been identified as phosphorylation sites. In the absence of the agonist, a basal phosphorylation of Ser(363) and Thr(370) was observed, whereas [D-Ala(2),Me-Phe(4),Gly (5)-ol]enkephalin (DAMGO)-induced receptor phosphorylation occurs at Thr(370) and Ser(**375**) residues. Furthermore, the role of these phosphorylation sites in regulating the internalization of MOR was investigated. The mutation of Ser(**375**) to Ala reduced the rate and extent of receptor internalization, whereas mutation of Ser(363) and Thr(370) to Ala accelerated MOR internalization kinetics. The present data show that the basal phosphorylation of MOR could play a role in modulating agonist-induced receptor internalization kinetics. Furthermore, even though μ-receptors and δ-opioid receptors have the same motif encompassing agonist-induced phosphorylation sites, the different agonist-induced internalization properties controlled by these sites suggest differential cellular regulation of these two receptor subtypes.

L13 ANSWER 15 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-09952 BIOTECHDS
TITLE: New cardiac actin gene comprising histidine to arginine or **glycine** to glutamic acid substitution, useful in the diagnosis of disease associated with the mutation, specifically idiopathic dilated cardiomyopathy; **recombinant** cardiac actin protein and nucleic acid used in idiopathic dilated cardiomyopathy diagnosis, therapy and gene therapy
AUTHOR: Keating M T; Olson T M
PATENT ASSIGNEE: Univ.Utah-Res.Found.
LOCATION: Salt Lake City, UT, USA.
PATENT INFO: US 6063576 16 May 2000
APPLICATION INFO: US 1998-106217 29 Jun 1998
PRIORITY INFO: US 1998-106217 29 Jun 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-375488 [32]
AB One of 2 isolated cardiac actin nucleic acids with a given DNA sequence, and encoding proteins with specific mutations, is claimed. Also claimed is a nucleic acid that hybridizes to the given DNA sequences, but not to the given 1,134 bp or 1,128 bp wild type DNA sequences. The claims also cover: a means of diagnosing the presence or absence of a mutation that causes an idiopathic dilated cardiomyopathy (IDC); a means of diagnosing the presence or absence of a mutation in cardiac actin; cells transfect by the given mutant DNA sequences; a vector containing a nucleic acid encoding a mutant cardiac actin; host cells transformed by that vector; and a pair of ss DNA primers used to detect the presence of a mutation in a cardiac actin gene. These can be used in diagnosis, prognosis and therapy of IDC, and in drug screening to identify compounds used to treat IDC. The 1st nucleic acid has a given 1,134 bp DNA sequence and encodes a given 377 protein sequence with an Arg317His or a Gly363Glu mutation. The 2nd nucleic acid has a given 1,128 bp DNA sequence and encodes a given **375** amino acid protein sequence with an Arg312His or Glu361Gly mutation. (36pp)

L13 ANSWER 16 OF 35 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1999278501 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10347236
TITLE: A regulatory domain (R1-R2) in the amino terminus of the N-methyl-D-aspartate receptor: effects of spermine, protons, and ifenprodil, and structural similarity to bacterial leucine/isoleucine/valine binding protein.
AUTHOR: Masuko T; Kashiwagi K; Kuno T; Nguyen N D; Pahk A J; Fukuchi J; Igarashi K; Williams K
CORPORATE SOURCE: Faculty of Pharmaceutical Sciences, Chiba University,

Chiba, Japan.
CONTRACT NUMBER: NS-35047 (NINDS)
SOURCE: Molecular pharmacology, (1999 Jun) 55 (6) 957-69.
Journal code: 0035623. ISSN: 0026-895X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990628
Last Updated on STN: 19990628
Entered Medline: 19990617

AB There are complex interactions between spermine, protons, and ifenprodil at N-methyl-D-aspartate receptors. Spermine stimulation may involve relief of proton inhibition, whereas ifenprodil inhibition may involve an increase in proton inhibition. We studied mutations at acidic residues in the NR1 subunit using voltage-clamp recording of NR1/NR2B receptors **expressed** in Xenopus oocytes. Mutations at residues near the site of the exon-5 insert, including E181 and E185, reduced spermine stimulation and proton inhibition. Mutation NR1(D130N) reduced sensitivity to ifenprodil by more than 500-fold, but had little effect on sensitivity to spermine and pH. Mutations at six other residues in this region of the NR1 subunit reduced the potency and, in some cases, the maximum effect of ifenprodil. These mutants did not affect sensitivity to pH, glutamate, **glycine**, or other hallmark properties of N-methyl-D-aspartate channels such as Mg2+ block and Ba2+ permeability. Residues in this region presumably form part of the ifenprodil-binding site. To model this region of NR1 we compared the predicted secondary structure of NR1 (residues 19-400) with the known structures of 1,400 proteins. This region of NR1 is most similar to bacterial leucine/isoleucine/valine binding protein, a globular amino acid binding protein containing two lobes, similar to the downstream S1-S2 region of glutamate receptors. We propose that the tertiary structure of NR1(22-**375**) is similar to leucine/isoleucine/valine binding protein, containing two "regulatory" domains, which we term R1 and R2. This region, which contains the binding sites for spermine and ifenprodil, may influence the downstream S1 and S2 domains that constitute the **glycine** binding pocket.

L13 ANSWER 17 OF 35 MEDLINE on STN
ACCESSION NUMBER: 1999306866 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10377256
TITLE: The DmpA aminopeptidase from Ochrobactrum anthropi LMG7991 is the prototype of a new terminal nucleophile hydrolase family.
AUTHOR: Fanuel L; Goffin C; Cheggour A; Devreese B; Van Driessche G; Joris B; Van Beeumen J; Frere J M
CORPORATE SOURCE: Laboratoire d'Enzymologie et Centre d'Ingenierie des Proteines, Universite de Liege, Institut de Chimie, B6, B-4000 Sart Tilman, Belgium.
SOURCE: Biochemical journal, (1999 Jul 1) 341 (Pt 1) 147-55.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 20000303
Entered Medline: 19990903

AB The DmpA (d-aminopeptidase A) protein produced by Ochrobactrum anthropi hydrolyses p-nitroanilide derivatives of **glycine** and d-alanine more efficiently than that of l-alanine. When regular peptides are utilized as substrates, the enzyme behaves as an aminopeptidase with a

preference for N-terminal residues in an l configuration, thus exemplifying an interesting case of stereospecificity reversal. The best-hydrolysed substrate is l-Ala-Gly-Gly, but tetra- and penta-peptides are also efficiently hydrolysed. The gene encodes a **375**-residue precursor, but the active enzyme contains two polypeptides corresponding to residues 2-249 (alpha-subunit) and 250-**375** (beta-subunit) of the precursor. Residues 249 and 250 are a Gly and a Ser respectively, and various substitutions performed by site-directed mutagenesis result in the production of an uncleaved and inactive protein. The N-terminal Ser residue of the beta-subunit is followed by a hydrophobic peptide, which is predicted to form a beta-strand structure. All these properties strongly suggest that DmpA is an N-terminal amidohydrolase. An exploration of the databases highlights the presence of a number of open reading frames encoding related proteins in various bacterial genomes. Thus DmpA is very probably the prototype of an original family of N-terminal hydrolases.

L13 ANSWER 18 OF 35 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998247296 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9572931
TITLE: Identification and analysis of genes involved in anaerobic toluene metabolism by strain T1: putative role of a **glycine** free radical.
AUTHOR: Coschigano P W; Wehrman T S; Young L Y
CORPORATE SOURCE: Department of Biological Sciences, Ohio University, Athens 45701-2979, USA.. Coschiga@ohiou.edu
SOURCE: Applied and environmental microbiology, (1998 May) 64 (5) 1650-6.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF036765
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980609
Last Updated on STN: 20000303
Entered Medline: 19980528

AB The denitrifying strain T1 is able to grow with toluene serving as its sole carbon source. Two mutants which have defects in this toluene utilization pathway have been characterized. A **clone** has been isolated, and subclones which contain tutD and tutE, two genes in the T1 toluene metabolic pathway, have been generated. The tutD gene codes for an 864-amino-acid protein with a calculated molecular mass of 97,600 Da. The tutE gene codes for a **375**-amino-acid protein with a calculated molecular mass of 41,300 Da. Two additional small open reading frames have been identified, but their role is not known. The TutE protein has homology to pyruvate formate-lyase activating enzymes. The TutD protein has homology to pyruvate formate-lyase enzymes, including a conserved cysteine residue at the active site and a conserved **glycine** residue that is activated to a free radical in this enzyme. Site-directed mutagenesis of these two conserved amino acids shows that they are also essential for the function of TutD.

L13 ANSWER 19 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1997-10430 BIOTECHDS
TITLE: Chemoenzymic synthesis of N-(phosphonomethyl)**glycine*****; **glyphosate precursor glyoxylic acid production using *****recombinant** Hansenula polymorpha or Pichia pastoris **expressing** 2-hydroxy-acid-oxidase and catalase, after cell permeabilization
AUTHOR: Gavagan J E; Fager S K; Seip J E; Clark D S; Payne M S; Anton D L; *DiCosimo R
CORPORATE SOURCE: Du-Pont
LOCATION: Central Research and Development Department, E.I. du Pont de

Nemours & Co., Experimental Station, P.O. Box 80328, Wilmington, DE 19880-0328, USA.
SOURCE: J.Org.Chem.; (1997) 62, 16, 5419-27
CODEN: JOCEAH
ISSN: 0022-3263
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Permeabilized resting cells of transformed methylotrophs Hansenula polymorpha GO1 or 13.168 and Pichia pastoris MSP10 or MSP8.6, **expressing** spinach (Spinacia oleracea) **recombinant** glycolate-oxidase (2-hydroxy-acid-oxidase, EC-1.1.3.15) and Saccharomyces cerevisiae **recombinant** catalase-T (EC-1.11.1.6) or endogenous catalase, were used for oxidation of glycolic acid with oxygen to obtain glyoxylic acid, in an aqueous mixture containing (aminomethyl)phosphonic acid (AMPA). Cells were permeabilized with cationic surfactant (e.g. benzyl cetyldimethylammonium chloride). In a typical reaction, 100 ml glycolic acid solution (0.500 M) with 0.375 M AMPA, 0.100 M isobutyric acid and 0.01 mM FMN, pH 8.3, was mixed with 5.0 g permeabilized strain 13.168, and agitated at 750 rpm with oxygen sparging at 5 deg. After 1.5 hr, 90.0% glyoxylic acid, 3.1% oxalic acid and 1.8% formic acid were obtained. It was possible to catalyze 30 consecutive batch reactions with enzyme recycle. The resulting solution was treated chemically to produce glyphosate, a herbicide. (40 ref)

L13 ANSWER 20 OF 35 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 97263736 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9109651
TITLE: Crystal structure of a polyhistidine-tagged **recombinant** catalytic subunit of cAMP-dependent protein kinase complexed with the peptide inhibitor PKI(5-24) and adenosine.
AUTHOR: Narayana N; Cox S; Shaltiel S; Taylor S S; Xuong N
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla 92093-0654, USA.
CONTRACT NUMBER: GM19301 (NIGMS)
RR01644 (NCRR)
SOURCE: Biochemistry, (1997 Apr 15) 36 (15) 4438-48.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970523
Last Updated on STN: 19980206
Entered Medline: 19970512

AB The crystal structure of the hexahistidine-tagged mouse **recombinant** catalytic subunit (H6-rC) of cAMP-dependent protein kinase (cAPK), complexed with a 20-residue peptide inhibitor from the heat-stable protein kinase inhibitor PKI(5-24) and adenosine, was determined at 2.2 A resolution. Novel crystallization conditions were required to grow the ternary complex crystals. The structure was refined to a final crystallographic R-factor of 18.2% with good stereochemical parameters. The "active" enzyme adopts a "closed" conformation as found in rC:PKI(5-24) [Knighton et al. (1991a,b) Science 253, 407-414, 414-420] and packs in a similar manner with the peptide providing a major contact surface. This structure clearly defines the subsites of the unique nucleotide binding site found in the protein kinase family. The adenosine occupies a mostly hydrophobic pocket at the base of the cleft between the two lobes and is completely buried. The missing triphosphate moiety of ATP is filled with a water molecule (Wtr 415) which replaces the gamma-phosphate of ATP. The **glycine**-rich loop between beta1 and beta2 helps to anchor the phosphates while the ribose ring is buried beneath beta-strand 2. Another ordered water molecule (Wtr **375**)

is pentacoordinated with polar atoms from adenosine, Leu 49 in beta-strand 1, Glu 127 in the linker strand between the two lobes, Tyr 330, and a third water molecule, Wtr 359. The conserved nucleotide fold can be defined as a lid comprised of beta-strand 1, the **glycine**-rich loop, and beta-strand 2. The adenine ring is buried beneath beta-strand 1 and the linker strand (120-127) that joins the small and large lobes. The C-terminal tail containing Tyr 330, a segment that lies outside the conserved core, covers this fold and anchors it in a closed conformation. The main-chain atoms of the flexible **glycine**-rich loop (residues 50-55) in the ATP binding domain have a mean B-factor of 41.4 A2. This loop is quite mobile, in striking contrast to the other conserved loops that converge at the active site cleft. The catalytic loop (residues 166-171) and the Mg2+ positioning loop (residues 184-186) are a stable part of the large lobe and have low B-factors in all structures solved to date. The stability of the **glycine**-rich loop is highly dependent on the ligands that occupy the active site cleft with maximum stability achieved in the ternary complex containing Mg x ATP and the peptide inhibitor. In this ternary complex the gamma-phosphate is secured between both lobes by hydrogen bonds to the backbone amide of Ser 53 in the **glycine**-rich loop and the amino group of Lys 168 in the catalytic loop. In the adenosine ternary complex the water molecule replacing the gamma-phosphate hydrogen bonds between Lys 168 and Asp 166 and makes no contact with the small lobe. This **glycine**-rich loop is thus the most mobile component of the active site cleft, with the tip of the loop being highly sensitive to what occupies the gamma-subsite.

L13 ANSWER 21 OF 35 MEDLINE on STN
ACCESSION NUMBER: 97033548 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8879245
TITLE: SOM 1, a small new gene required for mitochondrial inner membrane peptidase function in Saccharomyces cerevisiae.
AUTHOR: Esser K; Pratje E; Michaelis G
CORPORATE SOURCE: Botanisches Institut der Universitat Dusseldorf, Germany.
SOURCE: Molecular & general genetics : MGG, (1996 Sep 25) 252 (4) 437-45.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X90459
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 20000303
Entered Medline: 19961210

AB IMP1 encodes a subunit of the mitochondrial inner membrane peptidase responsible for the proteolytic processing of cytochrome oxidase subunit 2 (Cox2) and cytochrome b2 (Cytb2). The molecular defect in an imp1 mutation and the characterisation of a high-copy-number suppressor is described. A deletion of the suppressor region causes respiration deficiency. The DNA sequence revealed three very small overlapping ORFs. Constructs which carried termination codons within the ORFs or lacked ATG initiation codons still retained complementing activity on a high-copy-number plasmid. Nevertheless, the possibility that the suppressor acts at DNA or RNA level could be excluded. Subcloning of the ORFs, complementation analysis in low-copy-number plasmids and transcript mapping identified the 222 bp ORF as the suppressor gene designated SOM1. The SOM1 gene is transcribed into a **375** bp polyadenylated RNA and the deduced amino acid sequence predicts a small protein of 8.4 kDa with no significant sequence similarity to known proteins. In the som1 deletion mutant, proteolytic processing of the Cox2 precursor is prevented and Cytb2 is strongly reduced. SOM1 represents a new small gene which encodes a novel factor that is essential for the correct function of the Imp1 peptidase and/or the protein sorting machinery.

L13 ANSWER 22 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4

ACCESSION NUMBER: 1995:211524 BIOSIS
DOCUMENT NUMBER: PREV199598225824
TITLE: Synechococcus sp. PCC7942 Transformed with Escherichia coli bet Genes Produces **Glycine** Betaine from Choline and Acquires Resistance to Salt Stress.
AUTHOR(S): Nomura, Mika; Ishitani, Manabu; Takabe, Teruhiro; Rai, Ashwani K.; Takabe, Tetsuko [Reprint author]
CORPORATE SOURCE: BioSci. Cent., Nagoya Univ., Chikusa, Nagoya 464-01, Japan
SOURCE: Plant Physiology (Rockville), (1995) Vol. 107, No. 3, pp. 703-708.
CODEN: PLPHAY. ISSN: 0032-0889.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 May 1995
Last Updated on STN: 9 Jun 1995

AB Synechococcus sp. PCC7942, a fresh water cyanobacterium, was transformed by a shuttle plasmid that contains a 9-kb fragment encoding the Escherichia coli bet gene cluster, i.e. betA (choline dehydrogenase), betB (betaine aldehyde dehydrogenase), betI (a putative regulatory protein), and betT (the choline transport system). The **expression** of these genes was demonstrated in the cyanobacterial cells (bet-containing cells) by northern blot analysis, as well as by the detection of **glycine** betaine by 1H nuclear magnetic resonance in cells supplemented with choline. Endogenous choline was not detected in either control or bet-containing cells. Both control and bet-containing cyanobacterial cells were found to import choline in an energy-dependent process, although this import was restricted only to bet-containing cells in conditions of salt stress. **Glycine** betaine was found to accumulate to a concentration of 45 mM in bet-containing cyanobacterial cells, and this resulted in a stabilization of the photosynthetic activities of photosystems I and II, higher phycobilisome contents, and general protective effects against salt stress when compared to control cells. The growth of bet-containing cells was much faster in the presence of 0.**375** M NaCl than that of control cells, indicating that the transformant acquired resistance to salt stress.

L13 ANSWER 23 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5

ACCESSION NUMBER: 1995:415837 BIOSIS
DOCUMENT NUMBER: PREV199598430137
TITLE: Some studies on yeast cytosine permease.
AUTHOR(S): Acik, Leyla
CORPORATE SOURCE: Gazi Univ., Fac. Arts Sci., Dep. Biol., Ankara, Turkey
SOURCE: Turkish Journal of Biology, (1995) Vol. 19, No. 2, pp. 151-160.
ISSN: 1300-0152.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Sep 1995
Last Updated on STN: 1 Nov 1995

AB The nucleotide sequence of the mutant with altered cytosine permease activity in S. cerevisiae is determined. The cytosine permease genes from the mutant (LAH M3-2d) and the wild type (1300-14B) strains of S. cerevisiae were amplified by the PCR technique. **Cloning** of the cytosine permease gene (FCY2) fragments was successful and these fragments were sequenced. The mutant that was sequenced had four amino acid changes in the primary structure of the FCY2 gene encoding a protein of 533 amino acids: the residues 371 and **375** had been changed from isoleucine to valine; the residue 377, from asparagine to **glycine**; and the residue 447, from phenylalanine to isoleucine.

L13 ANSWER 24 OF 35 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 94237484 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8181752
TITLE: Characterization of the Escherichia coli gcv operon.
AUTHOR: Stauffer L T; Fogarty S J; Stauffer G V
CORPORATE SOURCE: Department of Microbiology, University of Iowa, Iowa City 52242.
CONTRACT NUMBER: GM-26878 (NIGMS)
SOURCE: Gene, (1994 May 3) 142 (1) 17-22.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L20872
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940621
Last Updated on STN: 19940621
Entered Medline: 19940610

AB The nucleotide (nt) sequence of the Escherichia coli gcvP gene was determined. The polypeptide deduced from the DNA sequence has an M(r) of 104,**375** (957 amino acids). In a minicell system, gcvP encodes a polypeptide that migrates at 93.3 kDa on sodium dodecyl sulfate-polyacrylamide gels. After the coding region, there is a 39-nt sequence followed by a T-rich sequence within which transcription appears to terminate. This region is preceded by a G/C-rich sequence that could form a stable stem-loop structure once transcribed, and is characteristic of Rho-independent transcription terminators. A Northern analysis identified an approx. 4700-nt RNA molecule, large enough to encode the T-, H-and P-proteins of the **glycine** cleavage enzyme complex. Analyses of gcvP::lacZ fusions with and without stop codons in gcvT, the first gene in the operon, confirmed gcvT, gcvH and gcvP lie in an operon. RNA slot blot analyses indicated that induction of gcv by **glycine** , and PurR-mediated repression of gcv occur at the level of transcription.

L13 ANSWER 25 OF 35 EMBASE
on STN DUPLICATE 7
ACCESSION NUMBER: 93206704 EMBASE
DOCUMENT NUMBER: 1993206704
TITLE: Sequences required for thrombomodulin cofactor activity within the fourth epidermal growth factor-like domain of human thrombomodulin.
AUTHOR: Lentz S.R.; Chen Y.; Sadler J.E.
CORPORATE SOURCE: Div. of Hematology-Oncology, Dept. of Internal Medicine, University of Iowa,Iowa City, IA 52242, United States
SOURCE: Journal of Biological Chemistry, (1993) 268/20 (15312-15317).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Activation of protein C by thrombin is stimulated by the endothelial cell cofactor thrombomodulin. The structural regions of thrombomodulin necessary for cofactor activity have been localized to the fourth through sixth epidermal growth factor (EGF)-like domains. The fourth EGF-like domain is unnecessary for high affinity thrombin binding, but is required for cofactor activity. To identify essential sequences within the fourth EGF-like domain, a series of **recombinant** human thrombomodulins consisting of EGF-like domains four through six were **expressed** in human kidney cells. These mutants contain replacements of disulfide loops within the fourth EGF-like domain, thereby conserving overall

disulfide bond structure. All of the mutants bound to thrombin with high affinity, and inhibited the fibrinogen-clotting activity of thrombin to a similar extent. Two regions of the fourth EGF-like domain were identified to be essential for cofactor activity: 1) the sequence consisting of amino acids Glu-357, Tyr-358, and Gln-359 shared by the overlapping first and second disulfide loops, and 2) the amino-terminal region of the third disulfide loop containing amino acids Glu-374, Gly-**375**, and Phe-376. These results suggest that amino acids critical for thrombomodulin cofactor activity are located near the junction between the two subdomains of the fourth EGF-like domain.

L13 ANSWER 26 OF 35 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:100414 HCAPLUS
DOCUMENT NUMBER: 120:100414
TITLE: 6-Phospho-β-galactosidases of Gram-positive and 6-phospho-β-glucosidase B of Gram-negative bacteria: Comparison of structure and function by kinetic and immunological methods and mutagenesis of the lacG gene of Staphylococcus aureus
AUTHOR(S): Witt, Elen; Frank, Rainer; Hengstenberg, Wolfgang
CORPORATE SOURCE: Abt. Biol., Ruhr-Univ. Bochum, Bochum, 4630, Germany
SOURCE: Protein Engineering (1993), 6(8), 913-20
CODEN: PRENE9; ISSN: 0269-2139
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 6-Phospho-β-galactosidase (I) of S. aureus, Lactococcus lactis, and Lactobacillus casei and 6-phospho-β-glucosidase B (II) of Escherichia coli build a subfamily inside a greater enzyme family, named glycosal hydrolase family 1, which in addition, contains 9 β-glycosidases of different origins. Kinetic and immunol. evidence is provided in this report which strengthens the relationship of the 4 6-phospho-β-glycosidases. It is shown that the I and II are able to split aromatic β-galactoside phosphates and β-glucoside phosphates. The turnover nos. of hydrolysis of substrates with different epimerization at C-4 of the glycon varied up to 15-fold only. Two polyclonal antisera, one derived against native I from S. aureus and the other derived against II, cross-reacted with both enzymes. Peptides of the proteins were separated by reverse phase HPLC. The cross-reacting peptides were sequenced and shown to be localized at almost the same position in the aligned primary structures of both enzymes. An insertion of 9 amino acids near these antigenic domains is unique for the 6-phospho-β-glycosidases and missing within the sequences of the β-glycoside-specific members of the family. The lacG gene of a I-neg. S. aureus mutant was **cloned** into E coli and sequenced. In the totally inactive mutant protein, the only substitution was Gly-332 changed to Arg. This amino acid was part of the sequence insertion near the antigenic domain reacting with both antisera. These data supported the assumption that this region is of great importance for the function of the enzymes and that it is possible dets. the specificity of the phosphorylated form of the substrates. In addition, I of S. aureus was modified by site-directed mutagenesis of the corresponding lacG gene in order to replace residues Glu-160 and Glu-**375**, which were suspected of being involved in the general acid catalysis of substrate hydrolysis, with Gln residues (mutant proteins E160Q and E375Q, resp.). Mutant protein E160Q showed a 1000-fold reduction in activity compared with wild-type I but retained some catalytic activity, suggesting that Glu-160 plays an important but not critical role in catalysis. Protein E375Q was found to be totally inactive consistent with the identification of Glu-**375** as the active site nucleophile of I of S. aureus. It was located in sequence motif -ENG- where Glu-358 was previously identified as the catalytically active nucleophile in β-glucosidase of Agrobacterium.

L13 ANSWER 27 OF 35 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 92129369 MEDLINE

DOCUMENT NUMBER: PubMed ID: 1733971
TITLE: Characterization of an abnormal fibrinogen Osaka V. with the replacement of gamma-arginine **375** by **glycine**. The lack of high affinity calcium binding to D-domains and the lack of protective effect of calcium on fibrinolysis.
AUTHOR: Yoshida N; Hirata H; Morigami Y; Imaoka S; Matsuda M; Yamazumi K; Asakura S
CORPORATE SOURCE: Institute of Hematology, Jichi Medical School, Tochigi, Japan.
SOURCE: Journal of biological chemistry, (1992 Feb 5) 267 (4) 2753-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920322
Last Updated on STN: 20000303
Entered Medline: 19920303

AB Prolonged thrombin time was completely corrected by the addition of millimolar concentrations of calcium in a new abnormal fibrinogen, Osaka V. Analysis of lysyl endopeptidase digests of A alpha-, B beta-, or gamma-chains by high performance liquid chromatography, and the following amino acid sequence analysis of relevant peptides revealed that about 50% of the gamma-chain has a replacement of gamma-arginine **375** by **glycine**. When fibrinogen was digested with plasmin in the presence of millimolar concentration of calcium, the amount of fragment D1 was about 50% of the normal control, and the rest was further cleaved to fragment D2, D3, or D62 with an apparent Mr of 62,000. Plasmic digestion of cross-linked fibrin in the presence of calcium resulted in the appearance of an abnormal fragment with an apparent Mr of 123,000 as well as fragments D2, D3, and D62, concomitant with the decrease of D dimer. The gamma-remnant of the abnormal fragment proved to be a cross-linked complex of the normal D1 gamma-remnant and residues 374-406/411 of the abnormal gamma-chain. The number of high affinity Ca(2+)-binding sites for the normal fibrinogen and fibrinogen Osaka V obtained by equilibrium dialysis was 2.88 (about 3) and 1.85, respectively, and that for the abnormal molecules was calculated as 0.9 (about 1) from their relative amounts in the samples, suggesting the lack of two Ca(2+)-binding sites in the D-domains. These data suggest that the normal structure of the COOH-terminal portion of the gamma-chain including residue **375** is required for the full **expression** of high affinity calcium binding to D-domains, the ability to be protected by calcium against plasmic digestion, and fibrin polymerization. During these studies, we found that the NH2-terminal amino acid of the gamma-remnant in fragments D or D dimer which were obtained after prolonged digestion with plasmin is gamma-Met89.

L13 ANSWER 28 OF 35 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1991:553821 HCAPLUS
DOCUMENT NUMBER: 115:153821
TITLE: Conformational stability of pig citrate synthase and some active-site mutants
AUTHOR(S): Zhi, Wang; Srere, Paul A.; Evans, Claudia T.
CORPORATE SOURCE: Pre-Clin. Sci. Unit, Dep. Veterans Aff. Med. Cent., Dallas, TX, 75216, USA
SOURCE: Biochemistry (1991), 30(38), 9281-6
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The conformational stabilities of native pig citrate synthase (PCS), a **recombinant** wild-type PCS, and six active-site mutant PCS were

studied in thermal denaturation expts. by CD and in urea denaturation expts. by using DTNB to measure the appearance of latent SH groups. His274 and Asp375 are conserved active-site residues in PCS that bind to substrates and are implicated in the catalytic mechanism of the enzyme. By site-directed mutagenesis, His274 was replaced with **glycine** (Gly) and arginine (Arg) while Asp375 was replaced with Gly, asparagine (Asn), glutamate (Glu), or glutamine (Gln). These modifications were previously shown to result in 103-104-fold redns. in enzyme specific activities. The thermal unfolding of PCS and the six mutants in the presence and absence of substrates showed large differences in the thermal stabilities of mutant proteins compared to the wild-type PCS. The functions of His274 and Asp375 in ligand binding were measured by oxalacetate protection against urea denaturation. Active-site mutations that decrease the specific activity of PCS also cause an increase in the conformational stability of the protein. Apparently, specific electrostatic interactions in the active site of citrate synthase are important in the catalytic mechanism in the chemical transformations as well as the conformational flexibility of the protein, both of which are important for the overall catalytic efficiency of the enzyme.

L13 ANSWER 29 OF 35 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 91161577 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2002038
TITLE: Isolation and sequence determination of cDNA encoding T-protein of the **glycine** cleavage system.
AUTHOR: Okamura-Ikeda K; Fujiwara K; Yamamoto M; Hiraga K; Motokawa Y
CORPORATE SOURCE: Institute for Enzyme Research, University of Tokushima, Japan.
SOURCE: Journal of biological chemistry, (1991 Mar 15) 266 (8) 4917-21.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M55448; GENBANK-M55449; GENBANK-M59799; GENBANK-M61694; GENBANK-M61695; GENBANK-M61696; GENBANK-M61697; GENBANK-M61698; GENBANK-M64236; GENBANK-M64721
ENTRY MONTH: 199104
ENTRY DATE: Entered STN: 19910505
Last Updated on STN: 19910505
Entered Medline: 19910417

AB T-protein is a component of the **glycine** cleavage system and catalyzes the tetrahydrofolate-dependent reaction. A partial cDNA for chicken T-protein was isolated by screening a chicken liver cDNA library with oligonucleotide probes based on amino acid sequences. The **clone** (CT5C) contains a 675-base pair insert encoding the C-terminal half of the T-protein. Screening of a bovine liver lambda gt10 cDNA library with the insert of CT5C as a probe detected a **clone** , BT5A, with an insert of about 2 kilobase pairs. The 1191-base pair coding region encodes a precursor protein of 397 amino acids (Mr 42,852) comprised of a 22-residue mitochondrial targeting sequence and a **375**-residue mature protein (Mr 40,534). The 33 amino acids immediately following the targeting sequence correspond exactly to those determined by sequence analysis of the amino terminus of the purified bovine T-protein. The mature protein contains several hydrophilic segments with a cluster of arginine and lysine. The T-protein cDNA probe hybridized to an mRNA species of about 2 kilobases in bovine brain, lung, heart, and liver. A probe for H-protein hybridized with two species of mRNA in these tissues and weak signals were also found in spleen. Although the enzymatic activities of T-protein and H-protein were found in these tissues where transcripts were found, activity of P-protein was

detected only in liver and brain. Southern blot analysis of genomic DNA suggested that T-protein is a single copy gene.

L13 ANSWER 30 OF 35 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 91:155241 SCISEARCH
THE GENUINE ARTICLE: FC217
TITLE: ISOLATION AND SEQUENCE DETERMINATION OF CDNA-ENCODING T-PROTEIN OF THE **GLYCINE** CLEAVAGE SYSTEM
AUTHOR: OKAMURAIKEDA K (Reprint); FUJIWARA K; YAMAMOTO M; HIRAGA K; MOTOKAWA Y
CORPORATE SOURCE: UNIV TOKUSHIMA, INST ENZYME RES, TOKUSHIMA 770, JAPAN (Reprint); TOYAMA MED & PHARMACEUT UNIV, SCH MED, DEPT BIOCHEM, SUGITANI, TOYAMA 93001, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991) Vol. 266, No. 8, pp. 4917-4921.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB T-protein is a component of the **glycine** cleavage system and catalyzes the tetrahydrofolate-dependent reaction. A partial cDNA for chicken T-protein was isolated by screening a chicken liver cDNA library with oligonucleotide probes based on amino acid sequences. The **clone** (CT5C) contains a 675-base pair insert encoding the C-terminal half of the T-protein. Screening of a bovine liver lambda-gt10 cDNA library with the insert of CT5C as a probe detected a **clone** , BT5A, with an insert of about 2 kilobase pairs. The 1191-base pair coding region encodes a precursor protein of 397 amino acids (M(r) 42,852) comprised of a 22-residue mitochondrial targeting sequence and a **375**-residue mature protein (M(r) 40,534). The 33 amino acids immediately following the targeting sequence correspond exactly to those determined by sequence analysis of the amino terminus of the purified bovine T-protein. The mature protein contains several hydrophilic segments with a cluster of arginine and lysine. The T-protein cDNA probe hybridized to an mRNA species of about 2 kilobases in bovine brain, lung, heart, and liver. A probe for H-protein hybridized with two species of mRNA in these tissues and weak signals were also found in spleen. Although the enzymatic activities of T-protein and H-protein were found in these tissues where transcripts were found, activity of P-protein was detected only in liver and brain. Southern blot analysis of genomic DNA suggested that T-protein is a single copy gene.

L13 ANSWER 31 OF 35 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1990:493937 HCAPLUS
DOCUMENT NUMBER: 113:93937
TITLE: Mutation of essential catalytic residues in pig citrate synthase
AUTHOR(S): Alter, Gerald M.; Casazza, Joseph P.; Zhi, Wang; Nemeth, Peter; Srere, Paul A.; Evans, Claudia T.
CORPORATE SOURCE: Southwest. Med. Cent., Univ. Texas, Dallas, TX, 75216, USA
SOURCE: Biochemistry (1990), 29(33), 7557-63
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two amino acid residues, histidine (His)274 and aspartate (Asp)**375** , were replaced singly in the active site of pig citrate synthase (PCS) with **glycine** (Gly)274, arginine (Arg)274, Gly375, asparagine (Asn)**375**, glutamate (Glu)**375**, and glutamine (Gln) **375**. The nonmutant protein and the mutant proteins were **expressed** in and purified from Escherichia coli, and the effects of these amino acid substitutions on the overall reaction rate and

conformation of the PCS protein were studied by initial velocity and full time course kinetic anal., behavior during affinity column chromatog., and monoclonal antibody reactivity. Native and mutant proteins purified similarly had a subunit mol. weight of 50,000 and were homologous when examined with 10 independent anti-PCS monoclonal IgGs or with a polyclonal anti-pig heart citrate synthase serum. No activity was detected for Asn375 or Gln375. The kcats of the other purified mutant proteins, however, were decreased by .apprx.103 compared to the nonmutant enzyme activity. The Km for oxalacetate was decreased 10-fold in the Glu375 protein and was reduced by half in Gly274 and Arg274 PCSs, while the Km for acetyl-CoA was decreased 2-3-fold in Gly274, Arg274, and Gln375 PCSa. A mechanism is proposed that electrostatically links His274 and Asp375.

L13 ANSWER 32 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 10

ACCESSION NUMBER: 1989:93944 BIOSIS
DOCUMENT NUMBER: PREV198987048080; BA87:48080
TITLE: EFFECTS OF 5' FLANKING SEQUENCES AND CHANGES IN THE 5' INTERNAL CONTROL REGION ON THE TRANSCRIPTION OF RICE GLYCYL TRANSFER RNA G-C-C GENE.
AUTHOR(S): REDDY P S [Reprint author]; PADAYATTY J D
CORPORATE SOURCE: DEP BIOCHEM, INDIAN INST SCI, BANGALORE-560 012, INDIA
SOURCE: Plant Molecular Biology, (1988) Vol. 11, No. 5, pp. 575-584.
CODEN: PMBIDB. ISSN: 0167-4412.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 6 Feb 1989
Last Updated on STN: 6 Feb 1989

AB A stretch of 71 nucleotides in a 1.2 kilobase pairs Pst I fragment of rice DNA was identified as tRNAGCCGLY gene by hibridization and nucleotide sequence analyses. The hybridization of genomic DNA with the tRNA gene showed that there are about 10 **glycine** tRNA genes per diploid rice genome. The 3' and 5' internal control regions, where RNA polymerase III and transcription factors bind, were found to be present in the coding sequence. The gene was transcribed into a 4S product in an yeast cell-free extract. The substitution of 5' internal control region with analogous sequences from either M13mp19 or M13mp18 DNA did not affect the transcription of the gene in vitro. The changes in three highly conserved nucleotides in the consensus 5' internal control region (RGYNNARYGG; R = purine, Y = pyrimidine, N = any nucleotide) did not affect transcription showing that these nucleotides are not essential for promotion of transcription. There were two 16 base pair repeats, 'TGTTTGTTTCAGCTTA' at -130 and -**375** positions upstream from the start of the gene. Deletion of 5' flanking sequences including the 16 base pair repeat at -**375** showed increased transcription indicating that these sequences negatively modulate the **expression** of the gene.

L13 ANSWER 33 OF 35 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1987-09626 BIOTECHDS
TITLE: Stable transformation of soybean by electroporation and root formation from transformed callus;
aminoglycoside-3'-phosphotransferase II gene **expression**; kanamycin-resistance
AUTHOR: Christou P; Murphy J E; Swain W F
CORPORATE SOURCE: Agracetus
LOCATION: Agracetus, 8520 University Green, Middleton, WI 53562, USA.
SOURCE: Proc.Natl.Acad.Sci.U.S.A.; (1987) 84, 12, 3962-66
CODEN: PNASA6
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Soybean (**Glycine** max) protoplasts from some commercially important cultivars have been genetically engineered using

electroporation with chimeric genes encoding kanamycin and antibiotic G418 resistance. Protoplasts were produced from zygotic embryos by enzyme treatment and electroporation was performed in Kao protoplast medium supplemented with 40 mM NaCl. The electric pulse was delivered via platinum wire electrodes and supplied by a 490-uF capacitor. An initial voltage of **375** V was used. The treated protoplasts were incubated at 0 deg for 5 min, diluted into Kao medium in flasks, and incubated in the dark at RT for 8 days. Kao cell culture medium was added dropwise and the flasks exposed to low light. The feeding process was repeated every 8-10 days. Microcalli were obtained after 2-3 wk. Electroporation of protoplasts with a chimeric aminoglycoside-3'-phosphotransferase II (APHII) gene and selection on media supplemented with kanamycin produced calli resistant to the antibiotic. Root formation was induced from transformed calli; the roots maintained APHII gene **expression**. (28 ref)

L13 ANSWER 34 OF 35 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1984:81343 HCAPLUS
DOCUMENT NUMBER: 100:81343
TITLE: Specific alterations of the EF-Tu polypeptide chain considered in the light of its three-dimensional structure
AUTHOR(S): Duisterwinkel, Feitse Jan; Kraal, Barend; De Graaf, J. Martien; Talens, Anneke; Bosch, Leendert; Swart, Guido W. M.; Parmeggiani, Andrea; La Cour, Troels F. M.; Nyborg, Jens; Clark, Brian F. C.
CORPORATE SOURCE: Dep. Biochem., State Univ. Leiden, Leiden, 2333 AL, Neth.
SOURCE: EMBO Journal (1984), 3(1), 113-20
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Specific alterations of the elongation factor Tu (EF-Tu) polypeptide chain were identified in a number of mutant species of this elongation factor. In 2 species, alanine-**375** (Ala-**375**), located on domain II, was found by amino acid anal. to be replaced by threonine and valine, resp. These replacements substantially lower the affinity of EF-Tu-GDP for the antibiotic kirromycin. Since kirromycin can be crosslinked to lysine-357, also located on domain II but structurally very far from Ala-**375**, these data suggest that the replacements alter the relative position of domains I and II. The Ala-**375** replacements also lower the dissociation rates of the binary complexes EF-Tu-GTP and the binding consts. for EF-Tu-GTP and phenylalanyl-tRNA. It is conceivable that these effects are also mediated by movements of domains I and II relative to each other. Replacement of **glycine**-222 (Gly-222) by aspartate (Asp) were found in another mutant by DNA sequence anal. of the **cloned** tufB gene, coding for this mutant EF-Tu. Gly-222 is part of a structural domain, characteristic for a variety of nucleotide binding enzymes. Its replacement by Asp does not abolish the ability of EF-Tu to sustain protein synthesis. It increases the dissociation rate of EF-Tu-GTP by .apprx.30%. In the presence of kirromycin, the mutant species of EF-Tu-GDP does not bind to the ribosome, in contrast to its wild-type counterpart. A possible explanation is now open for exptl. verification.

L13 ANSWER 35 OF 35 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1976:235817 BIOSIS
DOCUMENT NUMBER: PREV197662065817; BA62:65817
TITLE: **EXPRESSION** OF ANTIBIOSIS TO THE BOLLWORM IN 2 SOYBEAN GENOTYPES.
AUTHOR(S): BELAND G L; HATCHETT J H
SOURCE: Journal of Economic Entomology, (1976) Vol. 69, No. 4, pp. 557-560.
CODEN: JEENAI. ISSN: 0022-0493.
DOCUMENT TYPE: Article

FILE SEGMENT: BA
LANGUAGE: Unavailable

AB The mortality of Heliothis zea (Boddie) ranged from 55-100% when larvae were fed leaves of 2 resistant genotypes (PI 229358 and ED 73-**375**) of soybean, **Glycine** max (L.) Merr., in laboratory tests; mortality occurred primarily in 5th-8th instars. Other **expressions** of antibiosis demonstrated by the larvae included additional molts, reduced weight and longer developmental period than occurred when leaves of the susceptible cultivars, 'Bragg' and 'Davis,' were fed. Adult females from bollworm larvae fed PI 229358 and ED 73-**375** oviposited significantly fewer eggs than those from larvae fed 'Bragg' and 'Davis,' but the number of spermatophores per female, the lengths of the oviposition period and the total longevities were similar for females from the 4 diets. Adult males from larvae fed the resistant genotypes lived significantly longer than those from larvae fed the susceptible cultivars.

```
=> e wei m/au
E1            2     WEI LYNN WONG W/AU
E2            4     WEI LYNN X/AU
E3          806 --> WEI M/AU
E4            6     WEI M A/AU
E5           90     WEI M C/AU
E6           70     WEI M D/AU
E7            4     WEI M E/AU
E8            9     WEI M F/AU
E9            5     WEI M G/AU
E10         133     WEI M H/AU
E11           1     WEI M H Y/AU
E12           1     WEI M I/AU

=> s e3
L14         806 "WEI M"/AU

=> e difrancesco v/au
E1            1     DIFRANCESCO U/AU
E2            1     DIFRANCESCO U M/AU
E3           97 --> DIFRANCESCO V/AU
E4           16     DIFRANCESCO VALENTINA/AU
E5            1     DIFRANCESCOL/AU
E6            1     DIFRANCESO D/AU
E7            2     DIFRANCESO L/AU
E8            1     DIFRANCESO R/AU
E9            1     DIFRANCESO ROBIN/AU
E10           1     DIFRANCESSO L/AU
E11           6     DIFRANCIA C/AU
E12           4     DIFRANCIA CELENE/AU

=> s e3-e4
L15         113 ("DIFRANCESCO V"/AU OR "DIFRANCESCO VALENTINA"/AU)

=> e beasley e m/au
E1            1     BEASLEY E H/AU
E2            6     BEASLEY E L/AU
E3          300 --> BEASLEY E M/AU
E4            7     BEASLEY E O/AU
E5            1     BEASLEY E S G/AU
E6            2     BEASLEY E T/AU
E7            4     BEASLEY E W/AU
E8            2     BEASLEY E W 3RD/AU
E9            2     BEASLEY E W III/AU
E10           1     BEASLEY E W JR/AU
E11           1     BEASLEY EDWARD E/AU
```

```
E12          1     BEASLEY EDWARD EVANS/AU

=> s e3
L16        300 "BEASLEY E M"/AU

=> d his

     (FILE 'HOME' ENTERED AT 13:24:46 ON 30 JUL 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 13:25:29 ON 30 JUL 2004
L1      1222343 S KINASE?
L2      2111610 S CALCIUM OR CALMODULIN
L3        21392 S L1(4W)L2
L4           34 S HUMAN (A)L3
L5           28 DUP REM L4 (6 DUPLICATES REMOVED)
L6      6635055 S CLON? OR EXPRESS? OR RECOMBINANT
L7         5864 S L3 AND L6
L8         1850 S HUMAN AND L7
L9         1641 S RETINOBLASTOMAS
L10           1 S L8 AND L9
L11         331 S "375" AND GLYCINE
L12          64 S L6 AND L11
L13          35 DUP REM L12 (29 DUPLICATES REMOVED)
                E WEI M/AU
L14         806 S E3
                E DIFRANCESCO V/AU
L15         113 S E3-E4
                E BEASLEY E M/AU
L16         300 S E3

=> s l14 or l15 or l16
L17       1130 L14 OR L15 OR L16

=> s l3 and l17
L18          1 L3 AND L17

=> d all

L18   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AN    2002-17807  BIOTECHDS
TI    Nucleic acid molecules encoding calcium/calmodulin-dependent protein
      kinases, useful for preventing diagnosing and treating e.g. cancers,
      psoriasis and inflammation;
         recombinant protein production by vector-mediated gene transfer and
         expression in host cell, useful for gene therapy
AU    YE J; YAN C; DI FRANCESCO V; BEASLEY E M
PA    PE CORP NY
PI    US 6387677 14 May 2002
AI    US 2001-800960 8 Mar 2001
PRAI  US 2001-800960 8 Mar 2001
DT    Patent
LA    English
OS    WPI: 2002-478444 [51]
AB    DERWENT ABSTRACT:
      NOVELTY - An isolated nucleic acid molecule (I) encoding a
      calcium/calmodulin-dependent protein kinase, is new.
           DETAILED DESCRIPTION - An isolated nucleic acid molecule (I)
      encoding a calcium/calmodulin-dependent protein kinase, comprising a
      nucleotide sequence selected from: (a) a nucleotide sequence that encodes
      a protein comprising a fully defined 565 amino acid sequence (A1) given
      in the specification; (b) a nucleotide sequence comprising the fully
      defined 2061 nucleotide sequence (N1) given in the specification ((N1) is
      a complementary deoxyribonucleic acid (cDNA) encoding the kinase); and/or
```

(c) a nucleotide sequence comprising the defined 62804 nucleotide sequence (N2) given in the specification ((N2) is a genomic sequence that spans the gene encoding the kinase protein). INDEPENDENT CLAIMS are also included for: (1) a nucleic acid vector (II) comprising (I); (2) a host cell (III) containing the vector (II); (3) producing (IV) a polypeptide comprising (A1), comprising culturing the host cell (III) under conditions sufficient for the production of said polypeptide, and recovering said polypeptide from the host cell culture; and (4) an isolated nucleic acid molecule (I') comprising a nucleotide sequence that is completely complementary to (I).

BIOTECHNOLOGY - Preferred Vectors: The vector (II) is a plasmid, virus or bacteriophage. (I) is inserted into the vector in proper orientation and correct reading frame so that the protein of (A1) may be expressed by a cell transformed with the vector. The isolated nucleic acid molecule may be operatively linked to a promoter sequence. Preparation: (I) and the protein it encodes may be produced via standard recombinant and synthetic methodologies e.g. by culturing (IV) the cell (III) (claimed).

ACTIVITY - Cytostatic; Anti-inflammatory; Anti-arteriosclerotic; Anti-psoriatic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Protein therapy; Vaccine; Enzymatic (calcium/calmodulin-dependent protein kinase). The gene (I) and encoded protein are related to the family of calcium/calmodulin-dependent protein kinases, which are serine/threonine kinases. The protein shows a particularly high degree of similarity to calcium/calmodulin-dependent protein kinase II (CaM II). CaM II is comprised of alpha, beta, gamma, and delta subunits. Each subunit is encoded by a separate gene and alternatively splice forms of each subunit have been found (Breen et al., Biochem. Biophys. Res. Commun. 236 (2), 473-478 (1997)). CaM II exerts important effects on hormones and neurotransmitters that utilize calcium as a second messenger. Beta-cell CaM II activity is associated with insulin secretion, and multiple isoforms of CaM II are expressed in human islets of Langerhans (Breen et al., Biochem. Biophys. Res. Commun. 236 (2), 473-478 (1997)). It has been suggested that CaM II controls activation-induced cellular differentiation, and is important for imparting antigen-dependent memory to T cells (Bui et al., Cell 100: 457-467, 2000).

USE - These polynucleotide sequences (I) and the peptides they encode can be used as models for the development of human therapeutic targets, aid in the identification of therapeutic proteins, and serve as targets for the development of human therapeutic agents that modulate kinase activity in cells and tissues that express the **kinase**. The **calcium/calmodulin**-dependent protein kinase encoded by (I) is expressed in humans in the placenta, breast cancers (including mammary adenocarcinoma), skin melanotic melanomas, ovary adenocarcinomas, uterus leiomyosarcomas, Burkitt's lymphomas (lymph), duodenal adenocarcinomas (small intestine), and fetal brain tumors and in disease conditions including inflammation, arteriosclerosis, and psoriasis (claimed).

ADMINISTRATION - Standard methodologies.

ADVANTAGE - Kinase proteins, particularly members of the calcium/calmodulin-dependent protein kinase subfamily, are a major target for drug action and development. Accordingly, it is valuable to the field of pharmaceutical development to identify and characterize previously unknown members of this subfamily of kinase proteins. (I) Encodes a previously unidentified human kinase protein that has homology to members of the calcium/calmodulin-dependent protein kinase subfamily.

EXAMPLE - No suitable example given.(85 pages)

CC THERAPEUTICS, Protein Therapeutics; GENETIC TECHNIQUES and APPLICATIONS, Gene Expression Techniques and Analysis; DISEASE, Cancer; DISEASE, Cardiovascular; DISEASE, Other Diseases; PHARMACEUTICALS, Vaccines; DIAGNOSTICS, Molecular Diagnostics; THERAPEUTICS, Gene Therapy; DISEASE, Autoimmune Disease

CT RECOMBINANT CALCIUM, CALMODULIN-DEPENDENT PROTEIN-KINASE PREP., VIRUS,

PHAGE, PLASMID VECTOR-MEDIATED GENE TRANSFER, EXPRESSION IN HOST CELL, APPL. CANCER, PSORIASIS, INFLAMMATION, ARTERIOSCLEROSIS, THERAPY, GENE THERAPY, DIAGNOSIS, VACCINE, DRUG TARGET, DRUG DEVELOPMENT ENZYME EC-2.7.1.37 DNA SEQUENCE PROTEIN SEQUENCE TUMOR CYTOSTATIC ANTIINFLAMMATORY ANTIARTERIOSCLEROTIC (21, 48)

=> d his

(FILE 'HOME' ENTERED AT 13:24:46 ON 30 JUL 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 13:25:29 ON 30 JUL 2004

L1	1222343	S	KINASE?
L2	2111610	S	CALCIUM OR CALMODULIN
L3	21392	S	L1(4W)L2
L4	34	S	HUMAN (A)L3
L5	28	DUP REM	L4 (6 DUPLICATES REMOVED)
L6	6635055	S	CLON? OR EXPRESS? OR RECOMBINANT
L7	5864	S	L3 AND L6
L8	1850	S	HUMAN AND L7
L9	1641	S	RETINOBLASTOMAS
L10	1	S	L8 AND L9
L11	331	S	"375" AND GLYCINE
L12	64	S	L6 AND L11
L13	35	DUP REM	L12 (29 DUPLICATES REMOVED)
		E	WEI M/AU
L14	806	S	E3
		E	DIFRANCESCO V/AU
L15	113	S	E3-E4
		E	BEASLEY E M/AU
L16	300	S	E3
L17	1130	S	L14 OR L15 OR L16
L18	1	S	L3 AND L17

	Issue Date	Pages	Document ID	Title
1	20040708	72	US 20040132152 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
2	20040708	58	US 20040132053 A1	Sphingosine kinase enzyme
3	20040527	85	US 20040101885 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
4	20040506	63	US 20040086926 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
5	20040429	23	US 20040082581 A1	Substituted triazine kinase inhibitors
6	20040422	59	US 20040077699 A1	Substituted triazole diamine derivatives as kinase inhibitors
7	20040415	337	US 20040072160 A1	Molecular toxicology modeling
8	20040226	52	US 20040038363 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
9	20040205	63	US 20040022764 A1	Inhibition of microcompetition with a foreign polynucleotide as treatment of chronic disease
10	20040122	14	US 20040014112 A1	Novel human kinase proteins and polynucleotides encoding the same
11	20040108	45	US 20040006094 A1	4-pyrimidinamine derivatives, pharmaceutical compositions and related methods

	Issue Date	Pages	Document ID	Title
12	20040108	165	US 20040005560 A1	Novel full-length cDNA
13	20031225	50	US 20030235915 A1	Human-nucleic acid sequences from breast tumor tissue
14	20031204	34	US 20030225085 A1	2-pyridinamine compositions and related methods
15	20031113	47	US 20030212079 A1	4-pyrimidinamine derivatives, pharmaceutical compositions and related methods
16	20030918	45	US 20030175926 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
17	20030731	32	US 20030144315 A1	Novel polyketide derivatives
18	20030724	61	US 20030140354 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
19	20030605	168	US 20030104358 A1	Diagnosis methods based on microcompetition for a limiting GABP complex
20	20030501		US 20030082674 A1	HUMAN PYRIMIDINE RECEPTOR
21	20030424		US 20030078280 A1	Macroheterocylic compounds useful as kinase inhibitors

	Issue Date	Pages	Document ID	Title
22	20030424	39	US 20030077799 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
23	20030403	14	US 20030064495 A1	Novel human kinase proteins and polynucleotides encoding the same
24	20030327		US 20030060629 A1	Pyrazine derivatives as modulators of tyrosine kinases
25	20030320		US 20030053994 A1	Redirection of cellular immunity by protein tyrosine kinase chimeras
26	20030306		US 20030046726 A1	Synthetic DNA sequence having enhanced insecticidal activity in maize
27	20030123		US 20030017970 A1	Wound healing compositions
28	20030109		US 20030008883 A1	4-Pyrimidinamine derivatives, pharmaceutical compositions and related methods
29	20021226		US 20020198219 A1	2-pyridinamine compositions and related methods
30	20021205		US 20020183348 A1	Novel polyketide derivatives
31	20021205		US 20020182726 A1	Calcium-independent negative regulation by CD81 of receptor signalling
32	20021128		US 20020176851 A1	Redirection of cellular immunity by protein-tyrosine kinase chimeras
33	20021121		US 20020173036 A1	Cell line and method of making and using same

	Issue Date	Pages	Document ID	Title
34	20021003		US 20020142988 A1	G-coupled receptor showing selective affinity for ATP
35	20021003	94	US 20020142436 A1	Human adenylate cyclase and use therefor
36	20021003		US 20020142430 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
37	20021003		US 20020142429 A1	Elongation factor-2 kinase (EF-2 kinase) and methods of use therefor
38	20021003	40	US 20020142427 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
39	20020919	89	US 20020132325 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
40	20020905		US 20020123121 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
41	20020829		US 20020120013 A1	Regulation of phospholipase D activity
42	20020808		US 20020107289 A1	Regulation of phospholipase D activity
43	20020704		US 20020086391 A1	ISOLATED HUMAN KINASE PROTEINS, NUCLEIC ACID MOLECULES ENCODING HUMAN KINASE PROTEINS, AND USES THEROF

	Issue Date	Pages	Document ID	Title
44	20020530		US 20020065295 A1	Novel polyketide derivatives
45	20020321		US 20020035734 A1	G-COUPLED RECEPTOR SHOWING SELECTIVE AFFINITY FOR ATP
46	20020207		US 20020015987 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
47	20020124		US 20020009772 A1	NOVEL HUMAN CALCIUM CHANNELS AND RELATED PROBES, CELL LINES AND MEHTODS
48	20020110		US 20020004210 A1	CALCIUM-INDEPENDENT NEGATIVE REGULATION BY CD81 OF RECEPTOR SIGNALLING
49	20011213		US 20010051184 A1	METHOD FOR USING SOLUBLE CURCUMIN TO INHIBIT PHOSPHORYLASE KINASE IN INFLAMMATORY DISEASES
50	20040504		US 6730480 B1	Sphingosine kinase enzyme
51	20040413		US 6720488 B2	Transgenic maize seed and method for controlling insect pests

	Issue Date	Pages	Document ID	Title
52	20040406	59	US 6716604 B2	Nucleic acid molecules encoding a subunit of a human calcium/calmodulin-dependent protein kinase
53	20040323		US 6710048 B2	Pyrazine derivatives as modulators of tyrosine kinases
54	20040106		US 6673901 B2	Artificial antibody polypeptides
55	20031230	60	US 6670162 B2	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
56	20031216	81	US 6664085 B2	Isolated human calcium/calmodulin (CaMk) dependent kinase proteins
57	20031125	49	US 6653116 B2	Isolated human kinase proteins
58	20030923		US 6624302 B2	Polyketide derivatives
59	20030812		US 6605589 B1	Cathepsin inhibitors in cancer treatment

	Issue Date	Pages	Document ID	Title
60	20030805		US 6602698 B2	Human kinase proteins and polynucleotides encoding the same
61	20030527		US 6569867 B2	Polyketide derivatives
62	20021203		US 6489125 B1	Methods for identifying chemical compounds that inhibit dissociation of FKBP12.6 binding protein from type 2 ryanodine receptor
63	20021008	49	US 6461846 B2	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
64	20020924		US 6455501 B1	Wound healing compositions
65	20020903		US 6444646 B1	Use of angiotensin II type 2 receptor agonists in tissue repair
66	20020820		US 6436656 B1	Method for screening a test compound for potential as an immunosuppressive drug candidate
67	20020730	60	US 6426206 B1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
68	20020723		US 6423501 B2	Calcium-independent negative regulation by CD81 of receptor signaling

	Issue Date	Pages	Document ID	Title
69	20020625		US 6410014 B1	Redirection of cellular immunity by protein-tyrosine kinase chimeras
70	20020611		US 6403352 B1	Compositions and methods for production of male-sterile plants
71	20020521		US 6392013 B1	Redirection of cellular immunity by protein tyrosine kinase chimeras
72	20020514		US 6387677 B1	Nucleic acid molecules encoding human calcium/calmodulin (CaMK) dependent kinase proteins
73	20020326		US 6362395 B1	Compositions and methods for production of male-sterile plants
74	20020305		US 6353026 B1	Regulation of phospholipase D activity
75	20020212		US 6346406 B1	Elongation factor-2 kinase (EF-2 kinase), and methods of use therefor
76	20010710		US 6258776 B1	Calcium-regulated kinase
77	20010306	95	US 6197581 B1	Human adenylate cyclase and use therefor
78	20001226		US 6165978 A	Wound healing composition

	Issue Date	Pages	Document ID	Title
79	20001212		US 6160106 A	Tumor suppressor genes, proteins encoded thereby and use of said genes and proteins
80	20000919		US 6121014 A	Method for producing a plant-optimized nucleic acid coding sequence
81	20000815		US 6103492 A	Polynucleotide encoding mu opioid receptor
82	20000620		US 6077991 A	Compositions and methods for production of male-sterile plants
83	20000613		US 6075185 A	Synthetic DNA sequence having enhanced insecticidal activity in maize
84	20000418		US 6051760 A	Synthetic DNA sequence having enhanced insecticidal activity in maize
85	20000321	19	US 6040164 A	Nucleic acids encoding nuclear Dbf2-related (Ndr) kinases
86	20000314		US 6037521 A	Transgenic mouse expressing an .beta.-Amyloid transgene

	Issue Date	Pages	Document ID	Title
87	20000125		US 6018104 A	Nucleic acid promoter fragment isolated from a plant tryptophan synthase alpha subunit (trpA) gene
88	19991221		US 6004811 A	Redirection of cellular immunity by protein tyrosine kinase chimeras
89	19991109	19	US 5981205 A	Nuclear Dbf2- related (Ndr) kinases
90	19991019		US 5968817 A	DNA encoding serotonin receptors
91	19990921		US 5955430 A	Use of angiotensin II fragments and analogs thereof in tissue repair
92	19990720		US 5925376 A	Method for treating psoriasis using selected phosphorylase kinase inhibitor and additional compounds
93	19990615		US 5912170 A	Redirection of cellular immunity by protein-tyrosine kinase chimeras
94	19990112		US 5859336 A	Synthetic DNA sequence having enhanced activity in maize
95	19981110		US 5834432 A	Use of angiotensin II Type 2 receptor agonists in tissue repair

	Issue Date	Pages	Document ID	Title
96	19980210		US 5716935 A	Use of antiotensin II analogs in tissue repair
97	19970513		US 5629292 A	Use of angiotensin III and analogs thereof in tissue repair
98	19970429		US 5625136 A	Synthetic DNA sequence having enhanced insecticidal activity in maize
99	19970429		US 5625122 A	Mouse having a disrupted lck gene

	Issue Date	Pages	Document ID	Title
1	20040708	72	US 20040132152 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
2	20040527	85	US 20040101885 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
3	20040506	63	US 20040086926 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
4	20040429	23	US 20040082581 A1	Substituted triazine kinase inhibitors
5	20040422	59	US 20040077699 A1	Substituted triazole diamine derivatives as kinase inhibitors
6	20040226	52	US 20040038363 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
7	20040219	68	US 20040033565 A1	Isolated human G-Protein coupled receptors, nucleic acid molecules encoding human GPCR proteins, and uses thereof
8	20030925	141	US 20030181710 A1	Death domain-containing receptor polynucleotides, polypeptides, and antibodies
9	20030724	61	US 20030140354 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
10	20030605	168	US 20030104358 A1	Diagnosis methods based on microcompetition for a limiting GABP complex

	Issue Date	Pages	Document ID	Title
11	20030424	39	US 20030077799 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
12	20030320	62	US 20030054402 A1	Methods and compositions for identifying receptor effectors
13	20030220	46	US 20030036089 A1	Isolated human G-protein coupled receptors, nucleic acid molecules encoding human GPCR proteins, and uses thereof
14	20030130	41	US 20030022232 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
15	20021121	46	US 20020172940 A1	Methods and reagents for isolating biologically active peptides
16	20021024	25	US 20020157119 A1	Identification of activated receptors and ion channels
17	20021003	52	US 20020142430 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
18	20021003	40	US 20020142427 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
19	20020919	89	US 20020132325 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof

	Issue Date	Pages	Document ID	Title
20	20020905	63	US 20020123121 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
21	20020704	63	US 20020086391 A1	ISOLATED HUMAN KINASE PROTEINS, NUCLEIC ACID MOLECULES ENCODING HUMAN KINASE PROTEINS, AND USES THEROF
22	20020620		US 20020077458 A1	Death domain-containing receptor polynucleotides, polypeptides, and antibodies
23	20020502		US 20020053091 A1	Isolated human G-protein coupled receptors, nucleic acid molecules encoding human GPCR proteins, and uses thereof
24	20020418		US 20020045191 A1	Inhibition of the SRC kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma
25	20020228		US 20020025536 A1	Methods and reagents for isolating biologically active antibodies
26	20020207		US 20020015987 A1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
27	20020124		US 20020009772 A1	NOVEL HUMAN CALCIUM CHANNELS AND RELATED PROBES, CELL LINES AND MEHTODS
28	20011004		US 20010026926 A1	Methods and compositions for identifying receptor effectors

	Issue Date	Pages	Document ID	Title
29	20040406	59	US 6716604 B2	Nucleic acid molecules encoding a subunit of a human calcium/calmodulin-dependent protein kinase
30	20031230	60	US 6670162 B2	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
31	20031216	81	US 6664085 B2	Isolated human calcium/calmodulin (CaMk) dependent kinase proteins
32	20031125	49	US 6653116 B2	Isolated human kinase proteins
33	20021119	46	US 6482935 B1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
34	20021008	49	US 6461846 B2	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof
35	20020730	60	US 6426206 B1	Isolated human kinase proteins, nucleic acid molecules encoding human kinase proteins, and uses thereof

	Issue Date	Pages	Document ID	Title
36	20020716		US 6420110 B1	Methods and reagents for isolating biologically active peptides
37	20020514		US 6387677 B1	Nucleic acid molecules encoding human calcium/calmodulin (CaMK) dependent kinase proteins
38	20010703		US 6255059 B1	Methods for identifying G protein coupled receptor effectors
39	20010227		US 6194632 B1	Mouse model for congestive heart failure
40	20001212		US 6159705 A	Recombinant yeast cells for identifying receptor effectors

	L #	Hits	Search Text
1	L1	50012	kinase$2
2	L2	314302	calcium or calmodulin
3	L3	1045	l1 adj5 l2
4	L4	646505	clon$3 or express$3 or recombinant
5	L5	330	l3 same l4
6	L6	99	human same l5
7	L7	1550	neuroblastomas
8	L8	0	l6 same l7
9	L9	3392	BEASLEY
10	L10	20411	WEI DIFRANCESCO
11	L11	23623	l9 or l10
12	L12	40	l5 and l11